201-14716

Crompton

Product Safety and Regulatory Affairs

August 27, 2003

Marianne L. Horinko
Acting Administrator
U.S. Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116

Attn: Chemical Right-To-Know Program

Dear Administrator Horinko,

Crompton Corporation is submitting the enclosed Robust Summary and Test plan to the US EPA HPV chemical Challenge Program for the following chemical:

Phenol, 2-sec-butyl-4,6-dintro- (CAS # 88-85-7).

If you have any questions, please contact me at 203-573-3390 or e-mail to mark_thomson@cromptoncorp.com

Sincerely

Dr. Mark A. Thomson
Manager, Toxicology & International Product Registration
Crompton Corporation
Middlebury, CT 06749
USA

Responsible Care®
A Public Commitment

201-14716A

HIGH PRODUCTION VOLUME (HPV) CHEMICAL CHALLENGE PROGRAM

TEST PLAN

For

4,6-Dinitrobutylphenol

CAS No. 88-85-7

Submitted to the US EPA

BY

Crompton Corporation.

Table of Contents

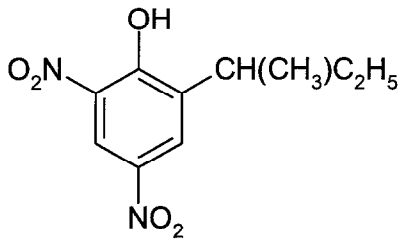
Test Plan for Dinoseb

1. General Information

1.1 CAS Number: 88-85-7

1.2 Molecular Weight: 240.2

1.3 Structure and formula: $C_{10}H_{12}N_2O_5$



1.4 Introduction

4,6-Dintrobutylphenol (DNBP) is used as a polymerization inhibitor for the production of styrene. Prior to 1990, DNBP was also used as a pre-emergence herbicide. Based on its toxicity, the use of DNBP as a herbicide is limited to certain government approved agricultural commodities.

2. Review of Existing Data and Development of Test Plan

Crompton Corporation has undertaken a comprehensive evaluation of all relevant data on the SIDS endpoints of concern for DNBP.

The availability of the data on the specific SIDS endpoints is summarized in Table 1. Table 1 also shows data gaps that will be filled by additional testing.

Table 1: Available adequate data and proposed testing on Dinoseb

CAS No. 10081-67-1	Information Available?	GLP	OECD Study?	Other Study?	Estimation Method?	Acceptable?	SIDS Testing required?
	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N
Physicochemical							
Melting Point	Y					Y	N
Boiling Point	Y					Y	N
Vapour Pressure	Y					Y	N
Water Solubility	Y					Y	N
Partition Coefficient (Kow)	Y					Y	N
Environmental Fate							
Biodegradation	Y				Y	Y	N
Hydrolysis	Y			Y		Y	N
Photodegradation	Y				Y	Y	N
Transport and Distribution between Environmental Compartments	Y				Y	Y	N
Ecotoxicology							
Acute Fish	Y	N	N	Y	N	Y	N
Acute Daphnia	Y	N	N	Y	N	Y	N
Acute Algae	Y	N	N	N	N	Y	N
Toxicology							
Acute Oral	Y	Y	N	Y	N	Y	N
Repeat Dose toxicity	Y	N	N	N	N	Y	N
Genetic toxicity – Gene mutation	Y	N				Y	N
Genetic toxicity – Chromosome aberration	N						Y
Reproductive toxicity	Y	N	N	N	N	Y	N
Developmental toxicity/teratogenicity	Y	N	N	Y/N	N	Y	N

A. Evaluation of Existing Physicochemical Data and Proposed Testing

1. Melting Point

The melting point is quoted as 38-42°C in a peer-reviewed publication

2. Boiling Point

The boiling point is quoted as 223°C in a peer-reviewed publication

3. Vapor Pressure

Values for vapor pressure quoted in the literature range between 2.3E-5 hPa and 7.0E-5 hPa at 30 – 20°C, respectively.

4. Water Solubility

The water solubility is quoted as 52 mg/L at 25°C in a peer reviewed publication.

5. Partition Coefficient

The Log Pow is quoted as 3.56 in a peer-reviewed publication.

Summary of Physicochemical Properties Testing: Existing data for melting point, boiling point, vapour pressure, partition coefficient and water solubility are considered to fill these endpoints adequately.

B. Evaluation of Existing Environmental Fate Data and Proposed Testing

1. Biodegradation

The biodegradability of the chemical has been estimated using Biowin v4.00 and the results indicate the chemical to not be readily biodegradable. The chemical contains no biodegradable groups, therefore no biodegradation testing is proposed.

2. Hydrolysis

A peer-reviewed report in the literature shows DNBP to be stable in aqueous solution over the pH range of 5-9.

3. Photodegradation

The potential for photodegradation of DNBP has been estimated using the AOPWIN v1.90, and indicated atmospheric oxidation via OH radicals reaction with a half-life of 31.8 hours.

4. Transport and Distribution between Environmental Compartments

An Epiwin Level III Fugacity Model calculation has been conducted DNBP and indicates distribution mainly to soil and, to a lesser extent, water for emissions of 1000 kg/hr simultaneously to air water and soil compartments.

Summary of Environmental Fate Testing: Existing data for photodegradation, hydrolysis, biodegradation and transport and distribution between environmental compartments are considered to fill these endpoints adequately.

C. Evaluation of Existing Ecotoxicity Data and Proposed Testing

1. Acute Toxicity to Fish

DNBP has been shown to be toxic to fish in several studies reported in the peer-reviewed literature (LC_{50} = 0.08 – 0.7 mg/L).

2. Acute Toxicity to Algae

DNBP has been shown to be toxic to algae (ErC_{50} = 4.3 - > 10 μM) in a study reported in the literature.

3. Acute Toxicity to Daphnia

DNBP has been shown to be toxic to daphnia (EC50 = 0.24 mg/L) in a peer-reviewed study reported in the literature.

4. Acute Toxicity to Bacteria (non-SIDS endpoint)

A value of EC50 > 6.4 mg/L is reported in the literature for this non-SIDS endpoint.

5. Chronic Toxicity to Fish (non-SIDS endpoint)

The $NOEC_{(weight)}$ for chronic effects on development and growth of fry in this study was < 0.5 μg/L. The concentration not affecting survival of the fry was between 4.9 and 10 μg/L.

Summary of Ecotoxicity Testing: DNBP is toxic to the aquatic environment. All SIDS endpoints have been filled adequately.

D. Evaluation of Existing Human Health Effects Data and Proposed Testing

1. Acute Oral Toxicity

The acute oral toxicity of a formulation containing DNBP has been examined in two studies conducted to GLP and following EPA OPP guidelines. In these studies the LD_{50} (rat) values ranged from 54.7 to 103.7 mg/kg bw.

2. Acute Inhalation Toxicity (non-SIDS endpoint)

In two GLP studies conducted to EPA OPP guidelines, the acute inhalation toxicity to rats of a formulation containing DNBP was found to be between0.033 and 0.29 mg/L (based on the active).

3. Acute Dermal Toxicity (non-SIDS endpoint)

In two GLP studies conducted using methods similar to EPA OPP guidelines, the acute dermal toxicity to rabbits of a formulation containing DNBP was found to be between 40 and 146 mg/kg bw.

4. Acute I.P. Toxicity (non-SIDS endpoint)

An LD_{50} (mouse) of 14.1 – 20.2 mg/kg is reported in the literature.

3. Eye Irritation (non-SIDS endpoint)

Formulations containing DNBP were highly irritating to the eyes of rabbits in two GLP studies performed to EPA OPP guidelines.

4. Repeat Dose Toxicity

In a combination subchronic feeding and single generation reproduction study, rats were fed DNBP for a total of 153 days at doses up to 500 ppm. The test groups dosed at 300 ppm and higher were terminated at 21 days due to mortality. In the remaining groups, growth was depressed monotonically. Blood alkaline phosphatase, alanine aminotransferase, potassium and BUN were significantly increased, while LDH and cholinesterase were depressed. Residue levels were dose dependent with blood > feces >urine > adipose >brain > liver. Aminopyrine N-demethylase activity was increased. Organ weights were decreased, while the organ weight/body weight ratios increased. A significant pathological change was diffuse tubular atrophy of the testes, particularly at 200 ppm.

Although only an abstract of the study is available in the literature, it is believed there is sufficient detail available to allow an assessment of repeated dose toxicity and no further studies are proposed to fulfil this endpoint.

5. Genotoxicity

DNBP tested negative in a number of Ames tests using Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA 100 and Escherichia coli strain WP2 uvrA-, both with and without metabolic activation (Arochlor-induced rat liver S9).

DNBP tested positive in an E. coli polA differential toxicity assay (E. coli strains p3478 & W3110) and in a S. typhimurium differential toxicity assay (S. typhimurium strains SL4700, SL4525, TA1978 and TA1538), both test conducted without metabolic activation. It also tested positive in a Bacillus subtilis recombination assay (B. subtilis strains M45 & H17) without metabolic activation, but was negative in a yeast gene mutation assay (S. cerevisiae D3) and in a UDS assay (Human Lung Fibroblast cells, strain WI-38) with and without metabolic activation.

In an in vivo Drosophila SLRL test using D. melanogaster, DNBP tested negative when administered to male fruit flies by oral feed.

6. Reproductive and Developmental Toxicity

In a one-generation fertility study DNBP was administered by oral feed to male Sherman rats for up to 77 days. At toxic dose levels the effects upon male reproduction are severe and appear dose related. At toxic dose levels histopathological changes in the gonads are observed that persist following withdrawal of treatment. The extent of the reproductive effects was seen to be beyond what was a consequence of dietary restriction alone. A NOEL of 3.8 mg/kg/day was established for both adult toxicity and reproductive effects, but the extent of the findings suggests that the effects on reproduction are not a consequence of a general systemic toxicity.

There are a number of developmental toxicity studies reported in the literature. In one study, the evaluation of postnatal offspring development following prenatal exposure was examined. Irrespective of the route of administration, the test material was shown to be a developmental

toxicant at dose levels that were toxic to the adult. The effects were either embryolethality/embryotoxicity, teratogenicity, fetotoxicity or a combination of effects. The nature of the findings suggest that developmental toxicity was not a consequence of toxicity to the adult. Some species variation in response was observed, but this was influenced by study design. In a study performed on rabbits following EPA guidelines, a NOEL for maternal and developmental toxicity of 1 mg/kg/day was established.

Summary of Human Health Effects Testing: An in vitro chromosome aberration test (OECD 473) will be performed. All other endpoints are considered to have been filled adequately.

3. Evaluation of Data for Quality and Acceptability

The collected data were reviewed for quality and acceptability following the general US EPA guidance [2] and the systematic approach described by Klimisch et al [3]. These methods include consideration of the reliability, relevance and adequacy of the data in evaluating their usefulness for hazard assessment purposes. This scoring system was only applied to ecotoxicology and human health endpoint studies per EPA recommendation [4]. The codification described by Klimisch specifies four categories of reliability for describing data adequacy. These are:

(1) Reliable without restriction: Includes studies or data complying with Good Laboratory Practice (GLP) procedures, or with valid and/or internationally accepted testing guidelines, or in which the test parameters are documented and comparable to these guidelines.

(2) Reliable with Restrictions: Includes studies or data in which test parameters are documented but vary slightly from testing guidelines.

(3) Not Reliable: Includes studies or data in which there are interferences, or that use non-relevant organisms or exposure routes, or which were carried out using unacceptable methods, or where documentation is insufficient.

(4) Not Assignable: Includes studies or data in which insufficient detail is reported to assign a rating, e.g. listed in abstracts or secondary literature.

4. References

[1] US EPA, EPI Suite Software, 2000

[2] USEPA (1998). Guidance for Meeting the SIDS Requirements (The SIDS Guide). Guidance for the HPV Challenge Program. Dated 11/2/98.

[3] Klimisch, H.-J., et al (1997). A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data. Regul. Toxicol. Pharmacol. 25:1-5

[4] USEPA (1999). Determining the Adequacy of Existing Data. Guidance for the HPV Challenge Program. Draft dated 2/10/99.

201-14716B

I U C L I D

Data Set

Robust Summaries

Existing Chemical	:	ID: 88-85-7
CAS No.	:	88-85-7
EINECS Name	:	dinoseb
EC No.	:	201-861-7
TSCA Name	:	Phenol, 2-(1-methylpropyl)-4,6-dinitro-
Molecular Formula	:	C10H12N2O5
Status	:	
Memo	:	DNBP Crompton Corporation US HPV
Printing date	:	26.06.2003
Revision date	:	
Date of last update	:	26.06.2003
Number of pages	:	1
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

2.1 MELTING POINT

Value	:	38 - 42 °C
Sublimation	:	
Method	:	
Year	:	1989
GLP	:	no data
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Reliability	:	(1) valid without restriction
12.02.2003		(3)

2.2 BOILING POINT

Value	:	223 °C
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Reliability	:	(1) valid without restriction
21.02.2003		(1)

Value	:	223 °C
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Reliability	:	(1) valid without restriction
21.02.2003		(8)

2.4 VAPOUR PRESSURE

Value	:	.0000227 hPa at 30 °C
Decomposition	:	
Method	:	other (measured):molecular beam
Year	:	1982
GLP	:	
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Reliability	:	(1) valid without restriction
21.02.2003		(30)

Value	:	.00007 hPa at 20 °C
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Reliability	:	(1) valid without restriction
21.02.2003		(22)

Value	:	1.33 hPa at 151.1 °C
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Reliability	:	(1) valid without restriction
21.02.2003		(34)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	3.56 at °C
pH value	:	
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Reliability	:	(1) valid without restriction
21.02.2003		(20)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	52 mg/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	
Year	:	1979
GLP	:	no data
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Reliability	:	(1) valid without restriction

21.02.2003 (35)

Solubility in	:	Water
Value	:	50 other: ppm at °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other: Gardner, K., J. Sci. Food. Agric., 7, 8, 1956
Year	:	1982
GLP	:	
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Reliability	:	(1) valid without restriction

21.02.2003 (30)

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Halflife t1/2	:	31.8 hour(s)
Degradation	:	% after
Quantum yield	:	
Deg. product	:	
Method	:	other (calculated): AOPWIN v 1.90
Year	:	2003
GLP	:	No
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Test condition	:	12 hr day, 1.56E6 OH/cm3
Reliability	:	(2) valid with restrictions

27.02.2003 (32)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	at °C
t1/2 pH9	:	at °C
Deg. product	:	no
Method	:	other: EPA Pesticides Assessment Guidelines (Oct. 18, 1982, Subdivision N, Series 161-1)
Year	:	1984
GLP	:	
Test substance	:	[^{14}C] 2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Purity: >99% Source: Uniroyal Chemical
Method	:	Two concentrations of [^{14}C]Dinoseb in aqueous buffered solutions of pH 5, 7 and 9 were incubated at 25°C. Products were monitored by thin layer chromatography (TLC) and liquid scintillation counting (LSC).
Result	:	After 30 days, Dinoseb showed no detectable hydrolysis products at any pH or concentration used. Dinoseb represented 96.3, 97.6 and 97.5% of the original radioactivity at pH 5, 7 and 9 (20 ppm), respectively, and 97.6, 97.5 and 97.7% of the original radioactivity at pH 5, 7 and 9 (40 ppm), respectively at 30 days. In general, the average radioactivity recovered from TLC was 83%. These data indicate that dinoseb is stable in aqueous solution over the pH 5-9 range.
Reliability	:	(2) valid with restrictions

02.04.2003 (6)

2.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	
Air	:	% (Fugacity Model Level I)
Water	:	% (Fugacity Model Level I)
Soil	:	% (Fugacity Model Level I)

Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: EPIWIN Level III Fugacity Model
Year : 2003
Test condition : Henry's Law Constant: 4.56E-7 atm-m3/mole (Henry Experimental database)
Vapor pressure: 5.26E-5 mm Hg (experimental)
Log Kow (KOWWIN experimental database)
Soil Koc: 1.49E+3 (calc by model)
Melting point: 40°C (experimental)

1000 kg/hr emissions to air, water and soil compartments.
Test substance : 2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)

	Mass Amount (percent)	Half-life (hr)	Emissions (kg/hr)
Air	1.66	63.6	1000
Water	26.7	900	1000
Soil	70.2	900	1000
Sediment	1.44	3.6E+3	0

	Fugacity (atm)	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	3.67E-11	398	366	13.3	12.2
Water	5.56E-12	452	588	15.1	19.6
Soil	4.52E-12	1.19E+3	0	39.6	0
Sediment	4.08E-12	6.08	0.632	0.203	0.0211

Persistence time: 734 hr
Reaction time: 1.08E+3 hr
Advection time: 2.31E+3 hr
Percent reacted: 68.2
Percent advected: 31.8

Half-lives (hr), (based upon Biowin (ultimate) and Aopwin):
Air: 63.65
Water: 900
Soil: 900
Sediment: 3600
Biowin estimate: 2.311 (weeks-months)

Advection times (hr):
Air: 100
Water: 1000
Sediment: 5E+4

Reliability : (2) valid with restrictions
27.02.2003 (32)

3.5 BIODEGRADATION

Type : aerobic
Inoculum :
Deg. product :
Method : other: Estimation using BIOWIN v4.00
Year : 2003
GLP :
Test substance : 2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)

Result : MITI Linear Biodegradation Probability: -0.2565
MITI Non-linear Biodegradation Probability: 0.0004

The substance is predicted to be not readily biodegradable

Reliability : (2) valid with restrictions
28.03.2003 (2)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	ca. .13 - .23
Limit test	:	no
Analytical monitoring	:	no
Method	:	other:ASTM E729-80
Year	:	1986
GLP	:	no
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Purity: >99%
Method	:	Test method used based on ASTM (1980) Standard Practice for conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians, ASTM Standard E729-80. American Society for Testing and Materials, Washington D.C. Statistical methods: Probit analysis; Finney, D.J. (1952), Statistical Methods in Biological Assay, Cambridge University Press, Cambridge, MA
Result	:	(1) 0.23 mg/l (2) 0.13 mg/l (3) 0.16 mg/l
Test condition	:	Test fish: No data Test conditions: Dilution water source: Pumped from the upper Saginaw Bay of Lake Huron off Whitestone Point and is limed and flocculated with ferrric chloride by the City of Midland Water Treatment Plant. The water was carbon filtered, u.v. irradiated and pH adjusted with carbon dioxide prior to use. Dilution water chemistry: hardness 77±4.3 mg/l, alkalinity 49±3.9 mg/l, conductivity 143±9.1 µmhos/cm. Over the study period the pH of the water ranged from 7.5 to 8.0. Stock solution preparation: Stock solution prepared by dissolving the appropriate amount of test material in acetone as a solvent carrier. Acetone levels did not exceed 0.5 ml/L during testing. The stability of the test material over the test period was examined prior to testing. Exposure vessel type: Round glass vessels, 25cm deep x 24.5 cm diameter Replicates: 0 Fish per replicate: 10 Water chemistry in test: (1) pH 7.0 - 7.4. Dissolved oxygen > 70% saturation. (2) pH 7.2 - 7.9. Dissolved oxygen > 52% saturation. (3) pH 7.3 - 7.9. Dissolved oxygen > 63% saturation. Test temperature range: (1) 17.0 - 17.2°C (2) 16.9 - 17.2°C (3) 17.0 - 17.3°C
Reliability	:	(2) valid with restrictions

04.03.2003 (16)

Type	:	static
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	= .088
Limit test	:	no
Analytical monitoring	:	yes
Method	:	other:
Year	:	1989
GLP	:	no
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Purity: 95%
Method	:	Test method used based on Peltier, W.H. & Weber, C.I., eds, Methods for measuring the acute toxicity of effluents to freshwater and marine organisms, EPA 600/4-85/013, US Environmental Protection Agency, Cincinnati, OH, 1985 Statistical methods: Litchfield, J.T & Wilcoxon, F.A., A simplified method of evaluating dose effect experiments, J. Pharmacol. Exp. Ther., 96, 99-113, 1949
Result	:	Confidence limits (95%) 0.078 - 0.098 mg/l
Test condition	:	Test fish: One year old fish used. Supplied by the LaCrosse National Fishery Research Laboratory Test conditions: Dilution water source: Reconstituted soft water Dilution water chemistry: hardness 44 mg/l, alkalinity 33 mg/l, pH 7.4. Stock solution preparation: Stock solutions were made by dissolving the chemical in 5 ml acetone, then diluting to volume with deionized water. Acetone concentrations did not exceed 0.1 ml/l in any exposure vessel. Test material stability: Recoveries from solution averaged 99% for the duration of the study. Stock solution concentrations and test vessel concentrations were 91 - 105% of the predicted concentrations. Exposure vessel type: 18 liter test vessel filled with 15 liters of reconstituted water. Replicates: 2 Fish per replicate: 10 Water chemistry in test: pH 7.4. Test temperature: 12°C
Reliability	:	(2) valid with restrictions

29.01.2003 (29)

Type	:	static
Species	:	Ictalurus punctatus (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	= .058
Limit test	:	no
Analytical monitoring	:	yes
Method	:	other:
Year	:	1989

GLP	:	no
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Purity: 95%
Method	:	Test method used based on Peltier, W.H. & Weber, C.I., eds, Methods for measuring the acute toxicity of effluents to freshwater and marine organisms, EPA 600/4-85/013, US Environmental Protection Agency, Cincinnati, OH, 1985 Statistical methods: Litchfield, J.T & Wilcoxon, F.A., A simplified method of evaluating dose effect experiments, J. Pharmacol. Exp. Ther., 96, 99-113, 1949
Result	:	Confidence limits (95%) 0.052 - 0.063 mg/l
Test condition	:	Test fish: One year old fish used. Supplied by the LaCrosse National Fishery Research Laboratory Test conditions: Dilution water source: Reconstituted soft water Dilution water chemistry: hardness 44 mg/l, alkalinity 33 mg/l, pH 7.4. Stock solution preparation: Stock solutions were made by dissolving the chemical in 5 ml acetone, then diluting to volume with deionized water. Acetone concentrations did not exceed 0.1 ml/l in any exposure vessel. Test material stability: Recoveries from solution averaged 99% for the duration of the study. Stock solution concentrations and test vessel concentrations were 91 - 105% of the predicted concentrations. Exposure vessel type: 18 liter test vessel filled with 15 liters of reconstituted water. Replicates: 4 Fish per replicate: 5 Water chemistry in test: pH 7.4. Test temperature: 12°C
Reliability 29.01.2003	:	(2) valid with restrictions (29)

Type	:	flow through
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	= .7
Limit test	:	no
Analytical monitoring	:	yes
Method	:	other:
Year	:	1984
GLP	:	no
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Supplier: Dow Chemical Co., Midland, MI Lot No.: AGR 133942 Purity: 98%
Method	:	No test guideline specified. Median lethal concentrations were determined on the pooled duplicates by the trimmed Spearman-Karber method: Hamilton, M.A., et al, Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays, Environ. Sci. Technol., 11, 714-719, 1977

Result	:	Confidence limits (95%) 0.6 - 0.7 mg/l
Test condition	:	Test fish: 30 day old fish from the stock culture at the Environmental Research laboratory, Duluth, MN (USEPA) were used. Test conditions: Dilution water source: Lake Superior water. Dilution water chemistry: hardness 47.6±2.4 mg/l, alkalinity 42.2±3.1 mg/l, acidity 2.0±0.2 mg/l, pH 7.5±0.1. Stock solution preparation: Water was delivered to the test tanks through a proportional diluter system with flow splitting chambers which provided five test material concentrations and a control, in duplicate. The diluter cycled every 6-12 minutes and delivered 0.5 liters of water alone or water plus test material to each aquarium. Stock solutions of test material were generated from sand columns. Concentrations used: mean concentrations over the test period were 0.24±0.02, 0.34±0.03, 0.50±0.07, 0.69±0.10 and 1.00±0.05 mg/l. Test material stability: Recoveries from solution averaged 99% for the duration of the study. Stock solution concentrations and test vessel concentrations were 91 - 105% of the predicted concentrations. Exposure vessel type: 17.5 liter glass aquaria (20 x 35 x 25 cm) containing 6 liters of water. Replicates: 2 Fish per replicate: 20 Water chemistry in test: Dissolved oxygen 92-99.3 % saturation Test temperature: 23.6 - 25.8°C
Reliability	:	(2) valid with restrictions
23.09.2002		(4)
Type	:	static
Species	:	other: Salmo clarki, Salvelinus namaycush
Exposure period	:	96 hour(s)
Unit	:	µg/l
LC50	:	32 - 1400
Limit test	:	no
Analytical monitoring	:	no
Method	:	other:
Year	:	1976
GLP	:	no
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Purity: Technical grade (95.8%) Source: Dow Chemical Company
Method	:	Committee on Methods for Toxicity Tests with Aquatic Organisms, Methods for acute toxicity tests with fish, macroinvertebrates and amphibians, US EPA, Washington, D.C. Ecol. Res. Ser. EPA-660/3-75-009: 67p, 1975 Statistical methods: 95% confidence intervals for LC50 were obtained from regression analysis of mortalities and concentrations (Litchfield, J.T. & Wllcoxon, E., A simplified method of evaluating dose-effect experiments, J. Pharacol. Exp. Ther., 96: 99-113, 1949).
Remark	:	The acute toxicity of the test substance was affected most by changes in pH. decreasing pH from 8.5 to 6.5 increased toxicity by factors of between

33 and 43. Toxicity of the test substance to the 2 species of fish increased with increases in water temperature and hardness. The influence of temperature and hardness on test substance toxicity was much less than that of pH, but was consistent for both species.

Test condition : Test fish: weight 0.3 - 1.6 g.

Test conditions:

Dilution water source: standard reconstituted water

Dilution water chemistry: pH 7.0-7.4, hardness 30-40 mg/liter (as CaCO3), alkalinity 40-50 mg/liter (as CaCO3). Water temperature 10°C.

Stock and test solution and how they are prepared: Stock solutions were prepared in acetone within the 2 weeks preceding treatment.

Stability of the test chemical solutions: Stability of the test solutions was shown by comparing the LC50 values of sulutions aged for 0 weeks and for 4 weeks prior to testing (Marking, L.L. & Dawson, V.K., The half life of biological activity of antimycin determined by fish bioassay, Trans. Am. Fish. Soc., 101, 100-105, 1972).

Exposure vessel type: no data

Number of replicates, fish/replicate: 1 replicate, 5 fish/replicate

Experiment 1 (temperature effects): pH 7.2, soft water, temperature 5, 10, 15°C

Experiment 2 (pH effects): temperature 10°C, soft water, pH 6.5, 7.5, 8.5

Experiment 3 (water hardness effects): temperature 10°C, pH 7.8, soft, hard, very hard water

Table 1: Acute toxicity of test substance to Salmo clarki and Salvelinus namaycush at different temperatures, pHs and water hardnesses.

Species	Temp (°C)	pH	Water hardness	96 hr LC50 (µg/L)	95% CI (µg/L)
		Temperature effects			
Salmo clarki	5	7.2	soft	58	48-70
	10	7.2	soft	67	56-81
	15	7.2	soft	42	32-54
Salvelinus namaycush	5	7.2	soft	135	102-178
	10	7.2	soft	44	38-51
	15	7.2	soft	36	24-54
		pH effects			
Salmo clarki	10	6.5	soft	41	34-49
	10	7.5	soft	130	89-189
	10	8.5	soft	1350	1160—1580
Salvelinus namaycush	10	6.5	soft	32	20-52
	10	7.5	soft	77	52-113
	10	8.5	soft	1400	1170-1680
		Water hardness effects			
Salmo clarki	10	7.8	soft	550	371-815
	10	7.8	hard	340	243-476
	10	7.8	very hard	280	207-317
Salvelinus namaycush	10	7.8	soft	280	205-383
	10	7.8	hard	140	104-189
	10	7.8	very hard	155	107-224

CI = confidence interval

Reliability : (2) valid with restrictions

13.11.2002 (36)

Type	:	static
Species	:	Ictalurus punctatus (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	.118
Limit test	:	
Analytical monitoring	:	no
Method	:	
Year	:	1977
GLP	:	no
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Method	:	Statistical methods: log-probit analysis used for LC50 calculation
Test condition	:	Test fish: One year old, average weight 14g, average length 12 cm. Test conditions: Water source: Non-chlorinated well water. Water chemistry: pH 8.2, alkalinity (CaCO3) 80 ppm, total solids 133 ppm, total soap hardness (CaCO3) 22 ppm, total acidity (CO2) 0 ppm, Iron 0.1 ppm.. Stock solution preparation: Stock solution prepared by dissolving the appropriate amount of test material in acetone as a solvent carrier. Acetone levels did not exceed 0.5 ml/L during testing. The stability of the test material over the test period was examined prior to testing. Exposure vessel type: 76 L all-glass aquaria Replicates: 0 Fish per replicate: 5 Water chemistry in test: no data Test temperature range: 20-21°C
Reliability	:	(4) not assignable

20.02.2003 (25)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	= .24
Analytical monitoring	:	no
Method	:	other:ASTM E729-80
Year	:	1986
GLP	:	no data
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Purity: >99%
Method	:	Test method used based on ASTM (1980) Standard Practice for conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians, ASTM Standard E729-80. American Society for Testing and Materials, Washington D.C. Statistical methods: Probit analysis; Finney, D.J. (1952), Statistical

Test condition	:	Methods in Biological Assay, Cambridge University Press, Cambridge, MA - Test organisms Source, breeding method: All daphnids used in this study were from a stock maintained for at least 3 generations and additionally were from the third or later brood. Age at study initiation: < 24 hours old Control group: water control and solvent carrier control - Test Conditions Stock solutions preparation: Stock solution prepared by dissolving the appropriate amount of test material in acetone as a solvent carrier. Acetone levels did not exceed 0.5 ml/L during testing. The stability of the test material over the test period was examined prior to testing. Test temperature range: 19.5 - 20.6°C Exposure vessel type: 250 ml glass beaker, filled to 200 ml. No aeration. Dilution water source: Pumped from the upper Saginaw Bay of Lake Huron off Whitestone Point and is limed and flocculated with ferrric chloride by the City of Midland Water Treatment Plant. The water was carbon filtered, u.v. irradiated and pH adjusted with carbon dioxide prior to use. Dilution water chemistry: hardness 77±4.3 mg/l, alkalinity 49±3.9 mg/l, conductivity 143±9.1 µmhos/cm. Over the study period the pH of the water ranged from 7.5 to 8.0. Lighting: 16 hour daylight/ 8 hour darkness Water chemistry in test: pH 7.6 - 8.2. Dissolved oxygen > 90% saturation. Element (unit) basis: Mortality Test design: 3 replicates (4 used for the high middle and low concentrations). 10 individuals per replicate.
Reliability	:	(2) valid with restrictions

05.11.2002 (16)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	other algae: Chlorella pyrenidosa, Chlorococcum sp., Lyngbya sp., Anabaena variabilis
Endpoint	:	growth rate
Exposure period	:	24 hour(s)
Unit	:	µmol/l
Limit test	:	
Analytical monitoring	:	no
Method	:	other:
Year	:	1977
GLP	:	no
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Source: unknown Purity: commercial grade
Method	:	Test organisms: Cultures of the 4 types of algae were obtained from Carolina Biological

Supply Co. and grown in Bold's basal medium with the pH at 6.6. Stock cultures were maintained at 25-27°C under continuous fluorescent light (3800 lx) and kept in uniform suspension by shaking at 80 cycles/min. The cultures were unialgal but not axenic.

Test Conditions:

Test temperature range: 24-26°C
Growth/test medium chemistry: no data
Dilution water source: no data
Exposure vessel type: 18x150 mm test tubes. Aerated.
Water chemistry in test (pH): no data
Stock solutions preparation: Bold's basal medium containing enough substance to give final concentrations of 0, 0.1, 1.0 and 10 µM after addition of algae.
Light levels and quality during exposure: Continuous fluorescent light (3200 lx).

Test design: 3 replicates/concentration
Concentrations used: 0, 0.1, 1.0 and 10 µM

Result : ErC50 values:

Chlorella: 4.3 µM
Chlorococcum: >10 µM
Lyngbya: 5.9 µM
Anabaena: >10 µM

Growth curves:

Chlorella: K = 89(0.1 µM), 89(1.0 µM), 0(10 µM)
Chlorococcum: K = 90(0.1 µM), 90(1.0 µM), 94(10 µM)
Lyngbya: K = 99(0.1 µM), 84(1.0 µM), 41(10 µM)
Anabaena: K = 101(0.1 µM), 99(1.0 µM), 99(10 µM)

K = growth rate in number of doublings per day

Reliability : (4) Not assignable
14.02.2003 (21)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type : aquatic
Species : Photobacterium phosphoreum (Bacteria)
Exposure period : 15 minute(s)
Unit : mg/l
EC50 : > 6.4
Analytical monitoring : no
Method : other: Microtox Test System, Microbics Corp, Carlsbad, CA

This test was conducted using the Microtox Test System, which utilizes a specially developed strain of the bioluminescent marine bacterium, Photobacterium phosporeum. The organisms are obtained as lyophilized cultures, each containing approximately one hundred milion cells.

Year : 1993
GLP : yes
Test substance : 2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Source: Uniroyal Chemical Company, Inc
Purity: >95%
Test condition : Test material concentration (nominal): 2.5, 5.0, 10.0, 20.0 mg/l

Replicates: 3

Remark	:	In this 15 minute static test, the test substance inhibited light output by the bacterium, however the concentration exceeded the apparent aqueous solubility of the test substance in diluent as undissolved test substance was observed in the stock solution. Therefore, the results obtained from this test may be exaggerated.
Reliabilty	:	(2) valid with restrictions

24.09.2002 (2)

4.5.1 CHRONIC TOXICITY TO FISH

Species	:	other: Salmo clarki, Salvelinus namaycush
Endpoint	:	other: mortality, growth and development
Exposure period	:	
Unit	:	µg/l
Method	:	American Public Health Association, American Water Works Association and Water Pollution Control Federation, Standard methods for the examination of water and waste water, 13th ed., New York, NY, 874p, 1971
Year	:	1976
GLP	:	no
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Purity: Technical grade (95.8%) Source: Dow Chemical Company
Test condition	:	Chronic Mortality: Type: Flow through Test concentrations: 0, 30, 46, 86, 210, 400 µg/L No. of fish/concentration: 30 of each species Test water: Spring water, 10°C, pH 7.4, hardness 162 mg/L (as CaCO3), alkalinity 206 mg/L (as CaCO3). Exposure period: Exposure continued beyond 96 hr until the mortality for 4 consecutive days was less than 5% of the fish remaining in each concentration. Toxicity was determined at 96 hr and at termination of exposure (10 days). Chronic Effects on Development and Growth: Type: Flow through Test concentrations: 0, 0.5, 1.6, 2.3, 4.9, 10 µg/L Fish species: Salvelinus namaycush No. of fish/ concentration: 25 Exposure: Eyed eggs were placed in each concentration of test substance 21 days before hatching. Exposure continued through 60 days after hatching. Analytical monitoring: The highest concentrations of test substance were analyzed by gas chromatography at 2, 4 and 6 weeks. Statistical methods: Length and weight of fry at 60 days analyzed using analysis of variance and multiple means comparison tests (least significant difference) to determine significant differences between treatments (Snedecor, G.W., Statistical methods, Iowa State University Press, Ames,

Iowa, 534p, 1965

Result : Chronic Mortality:

Salmo clarki: LC50 (96h) = 152 µg/L, LC50 (Terminal day) = 125 µg/L

Salvelinus namaycush: LC50 (96h) = 79µg/L, LC50 (Terminal day) = 65 µg/L

Chronic Effects on Development and growth:

See Table 1

NOEC (wt): < 0.5 µg/L

Table 1: Chronic effects on development and growth of Salvelinus namaycush

				Fry, 60-days posthatching		
Concn (µg/L)	Yolk absorption [a] (days)	Alevin survival (%)	Fry survival (%)	Survival (%)	Wt (mg)	Length (mm)
0	31	96	100	86	378	33
0.5	38	96	78	75	247 [b]	30 [b]
1.6	37	76	100	76	241 [b]	30 [b]
2.3	39	87	90	78	244 [b]	30 [b]
4.9	40	88	95	83	208 [b]	28 [b]
10.0	40	24	100	22	152 [b]	25 [b]

[a] Calculated from median hatch date to median yolk absorption date at 9.5 °C
[b] Significantly different from control group ($LSD_{0.05}$)

Remark : Chronic Mortality:

Exposure of the 2 fish species to the test substance over a period of 10 days did not cause cumulative mortality. Increased exposure time resulted only in slight decreases in LC50s.

Chronic Effects on Development and Growth:

Survival of Salvelinus namaycush fry in water containing 10 µg/L test substance was 22%.At concentrations of 4.9 µg/L or less, the survival of fry was similar to that of controls, but yolk absorption time was increased by 6-9 days. Growth of fry was sgnificantly (P<0.05) lower than that of control fish at all concentrations of test substance.

Although the concentration not affecting survival of the fry was between 4.9 and 10 µg/L, the test substance appears to affect growth of the fry at concentrations below 0.5 µg/L.

Reliability : (1) Valid without restrictions
13.11.2002 (36)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	= 54.7 - 92.08 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	50
Vehicle	:	water
Doses	:	Male/female: 40, 53, 69, 91, 120 mg/kg
Method	:	EPA OPP 81-1
Year	:	1980
GLP	:	yes
Test substance	:	DINOSEB 5 Source: Uniroyal Chemical Purity: 51 - 55%
Test condition	:	Age: No data Doses per time period: Single dose Concentration: 1.0 %w/v Post dose observation period: 14 days
Remark	:	Statistical methods: Knudsen, L.F. & Curtis, J.M., J. Am. Stat. Assoc., 42, 282, 1947
Result	:	LD50 confidence limits: Male: 86.19 - 98.42 mg/kg Female: 42.76 - 71.76 mg/kg Clinical signs at each dose level: Ataxia seen in 120 mg/kg males and 69, 91 and 120 mg/kg females Necropsy findings: No noteworthy findings

Table 1: Number of deaths at each dose level

Dose (mg/kg)	No. of deaths	
	Male	Female
40	0	0
53	0	1
69	0	5
91	2	4
120	5	5

Table 2: Time of death at each dose level

Dose (mg/kg)	Time of death (No./day)	
	Male	Female
40	0/0	0/0
53	0/0	1/2
69	0/0	2/1, 3/2
91	2/2	4/2
120	1/1, 4/2	5/2

Reliability : (1) valid without restriction
29.01.2003 (9)

Type	:	LD50
Value	:	= 89.93 - 103.72 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female

Number of animals	:	50
Vehicle	:	water
Doses	:	40, 53, 69, 91, 120 mg/kg
Method	:	EPA OPP 81-1
Year	:	1980
GLP	:	yes
Test substance	:	DINOSEB 3+1 Source: Uniroyal Chemical Purity: 51 - 55%
Remark	:	Statistical methods: Knudsen, L.F. & Curtis, J.M., J. Am. Stat. Assoc., 42, 282, 1947
Test condition		Age: No data Doses per time period: Single dose Concentration: 1.0 %w/v Post dose observation period: 14 days
Result	:	LD50 confidence limits: Male: 95.72 - 113.42 mg/kg Female: 84.15 - 96.05 mg/kg Clinical signs at each dose level: Deacreased activity seen in 91 and 120 mg/kg males and 120 mg/kg females. Ataxia seen in 120 mg/kg males and females. Urinary incontinence seen in 120 mg/kg females. Necropsy findings: No noteworthy findings

Table 1: Number of deaths at each dose level

Dose (mg/kg)	No. of deaths	
	Male	Female
40	0	0
53	0	0
69	0	0
91	1	3
120	4	5

Table 2: Time of death at each dose level

Dose (mg/kg)	Time of death (No./day)	
	Male	Female
40	0/0	0/0
53	0/0	0/0
69	0/0	0/0
91	1/1	3/1
120	4/1	3/1, 2/2

Reliability : (1) valid without restriction
24.09.2002 (9)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC50
Value	:	.2 - 2.19 mg/l
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	10
Vehicle	:	water
Doses	:	0.2, 2.19 mg/l nominal
Exposure time	:	4 hour(s)
Method	:	EPA OPP 81-3
Year	:	1980

GLP : yes
Test substance : DINOSEB 5
Source: Uniroyal Chemical
Purity: 51 - 55%
Test condition : Concentration: Measured concentrations of test substance at nominal concentrations of 0.2 and 2.19 mg/L were 0.033 mg/L and 0.29 mg/L, respectively.

Post dose observation period: 14 days

Remark : Statistical method: Finney, D.J., Statistical Methods in Biological Assay, 2nd edn, Griffin Press, London, 1971

Result : Clinical signs at each dose level: Approximately 1 hour post initiation of the exposure, animals in both the high and low dose groups exhibited labored breathing and decreased activity. In addition, animals in the high dose group exhibited nasal discharge soon after initiation. While the pharmatotoxic signs exhibited by the low dose animals cleared by day 3, the severity of signs in the high dose animal group increased up to the time of death.

Necropsy findings: There was no clear significant association between any of the necropsy findings and administration of the test material.

Table 1: Number of deaths at each dose level

Dose	No. of deaths	
(mg/L)	Male	Female
0.2	0	0
2.19	5	5

Table 2: Time of death at each dose level

Dose	Time of death (No./day)	
(mg/L)	Male	Female
0.2	0/0	0/0
2.19	3/0, 2/1	1/0, 4/1

Reliability : (1) valid without restriction
29.01.2003 (11)

Type : LC50
Value : .207 - 2.12 mg/l
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 10
Vehicle : water
Doses : 0.207, 2.12 mg/l nominal
Exposure time : 4 hour(s)
Method : EPA OPP 81-3
Year : 1980
GLP : yes
Test substance : DINOSEB 3+1
Source: Uniroyal Chemical
Purity: 51 - 55%
Test condition : Concentration: Measured concentrations of test substance at nominal concentrations of 0.207 and 2.12 mg/L were 0.035 mg/L and 0.13 mg/L, respectively.

Post dose observation period: 14 days

Remark : Statistical method: Finney, D.J., Statistical Methods in Biological Assay, 2nd edn, Griffin Press, London, 1971

Result : Clinical signs at each dose level: Approximately 1 hour after the initiation of the exposure, all the animals in the high dose group exhibited labored breathing and nasal discharge. In the low dose group only one animal showed an alteration of physical signs.

Necropsy findings: The findings indicate that the test material did not produce a significant toxicological effect. All findings were considered to be common findings in untreated rats.

Table 1: Number of deaths at each dose level

Dose (mg/L)	No. of deaths	
	Male	Female
0.207	0	0
2.12	4	5

Table 2: Time of death at each dose level

Dose (mg/L)	Time of death (No./day)	
	Male	Female
0.207	0/0	0/0
2.12	1/1, 3/2	5/1

Reliability : (1) valid without restriction
24.09.2002 (10)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD50
Value : 40 mg/kg bw
Species : rabbit
Strain : New Zealand white
Sex : male/female
Number of animals : 110
Vehicle :
Doses : 0, 16, 24, 38, 59, 92, 144, 225 mg/kg
Method : other: Similar to EPA OPP 81-2
Year : 1980
GLP : yes
Test substance : DINOSEB 5
Source: Uniroyal Chemical
Purity: 51 - 55%

Method : Prior to dosing, the fur was clipped from the dorsal surface of each animal. Six males and six females were assigned to each of seven treatment groups and one sham treated control group.

The exposed skin was abraded on 3 males and 3 females per group immediately prior to application of the test article, penetrating the stratum corneum but not the dermis.

The test article was applied over the clipped area of abraded and intact skin. to prevent evaporation and maximize contact of the test article, the trunk of each animal was wrapped with an occlusive binder. After the 24 hour exposure period the binders were removed and the sites wiped with

clean gauze. Protective collars were placed on all animals and not removed for 48 hours.

Result : LD50 confidence limits (95%): 33 – 49 mg/kg

Number of deaths at each dose level: see Table 1

Time of death: see Table 2

Clinical signs at each dose level:

Decreased activity, salivation, nasal discharge, increase in respiratory rate and mortality were the most frequently observed signs in both males and females belonging to the treatment groups.

Necropsy findings:

For male and female animals that died, a probable test material effect was noted in the stomach. Approximately 60% of these animals showed softening and thinning of large areas of the stomach wall.

Red areas of treated skin were occasionally seen in both abraded and non-abraded treated skin of males and females that died, but these observations were similarly seen in both sacrificed test survivors and controls. Also, lungs that were filled with pink foamy liquid were observed frequently in both sexes of animals that died, but this finding is common for untreated stock animals that die of non-respiratory causes.

Other gross findings of test animals consisted of expected color alterations. For instance, dark, red, mottled, pale, or blanched areas were occasionally observed in organs such as lungs, kidneys, spleen, and liver. Similar color changes were also seen in lungs and kidneys of control rabbits.

Microscopic examination of treated skin revealed a suggestive test material effect. That is, slight acute inflammation in the superficial layer of the dermis was observed more frequently in test animals (2 of 7 males, 1 of 6 females). These findings in the treated skin of test animals may be related to the test material which caused considerable mortality of test rabbits. However, the skin observations are also expected changes due to experimental methods of the study including clipping, abrading and occlusive bindings.

The most severe skin lesion was an ulcer (5 mm) with adjacent moderate dermal fibrosis, moderate epidermal thickening, and mild acanthosis, observed in a control male animal. Other incidental skin pathologic findings included mild crust formation and slight hyperkeratosis in one test animal, mild chronic inflammation in the superficial dermis of one test animal, and slight loss of hair follicles in one control rabbit.

Table 1: Number of deaths at each dose level

Dose (mg/kg)	No. of deaths	
	Male	Female
16	0	0
24	0	0
38	5	4
59	4	5
92	5	6
144	6	6
225	6	6

Table 2: Time of death at each dose level

Dose (mg/kg)	Time of death (No./day)	
	Male	Female
16	0/0	0/0
24	0/0	0/0
38	5/2	4/2
59	2/1, 2/2	1/1, 4/2
92	1/1, 4/2	4/1, 2/2
144	6/1	3/1, 3/2
225	6/1	2/1, 4/2

Reliability : (1) valid without restriction
29.01.2003 (14)

Type : LD50
Value : 146 mg/kg bw
Species : rabbit
Strain : New Zealand white
Sex : male/female
Number of animals : 74
Vehicle :
Doses : 0, 59, 92, 144, 225 mg/kg
Method : other: Similar to EPA OPP 81-2
Year : 1980
GLP : yes
Test substance : DINOSEB 3+1
Source: Uniroyal Chemical
Purity: 51 - 55%

Method : Prior to dosing, the fur was clipped from the dorsal surface of each animal. Six males and six females were assigned to each of four treatment groups and one sham treated control group.

The exposed skin was abraded on 3 males and 3 females per group immediately prior to application of the test article, penetrating the stratum corneum but not the dermis.

The test article was applied over the clipped area of abraded and intact skin. to prevent evaporation and maximize contact of the test article, the trunk of each animal was wrapped with an occlusive binder. After the 24 hour exposure period the binders were removed and the sites wiped with clean gauze. Protective collars were placed on all animals and not removed for 48 hours.

Result : LD50 confidence limits (95%): 92 - 256 mg/kg

Number of deaths at each dose level: see Table 1

Time of death: see Table 2

Clinical signs at each dose level:

The majority of animals in the four treatment groups experienced decreased activity (22 males and 24 females) and ataxia (21 males and 24 females).

Necropsy findings:

About half the animals that died showed thinning and softening of large

areas of the wall of the stomach and in 2 animals the wall was perforated. While autolysis does affect the stomach wall, in these animals the extent of softening and the friableness of the stomach wall seemed well beyond expected changes of autolysis.

Histopathalogical examination revealed no test effect on the test material treated skins.

Table 1: Number of deaths at each dose level

Dose (mg/kg)	No. of deaths	
	Male	Female
59	1	0
92	0	3
144	4	3
225	3	5

Table 2: Time of death at each dose level

Dose (mg/kg)	Time of death (No./day)	
	Male	Female
59	1/2	0/0
92	0/0	3/2
144	4/2	1/1, 2/2
225	3/2	2/1, 3/2

Reliability : (1) valid without restriction
19.09.2002 (15)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type : LD50
Value : 14.1 - 20.2
Species : mouse
Strain : Swiss Webster
Sex : female
Number of animals :
Vehicle :
Doses : 12.5 - 25 mg/kg
Route of admin. : i.p.
Exposure time :
Method :
Year : 1975
GLP : no
Test substance : 2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Purity: Technical grade
Lot #: 7200206
Supplier: Dow Chemical Co., Midland, MI
Method : Method: Nonpregnant female mice were given single ip injections. Mice were maintained under 3 sets of environmental conditions. For the high temperature condition mice were maintained in an air-circulating incubator at 32°C for 24 hr after treatment. The low temperature groups were kept at 6°C for 4 hr following treatment and thereafter at room temperature (23-24°C). Temperature control animals were maintained in the incubator at 25°C for 24 hr.

No of animals/dose: at least 5/group
Vehicle: The test material was dissolved in 0.1N NaOH and returned to physiological pH with 0.1N HCl.
Volume administered: 10mL/kg bw

Table 1: 24h LD50 for single ip injections of dinoseb in mice maintained at 25 or 32°C, or given a 4 hr exposure to 6°C following treatment

Environmental treatment (°C)	Δ Body temperature (mean±SE)[1]	LD50 (mg/kg)	Potency ratio
6	-5.6±0.8	22.5 (20.8-24.3)[2]	1.11 (1.01-1.22)[2]
25	-1.0±0.2	20.2 (18.9-21.6)	1.43 (1.19-1.72)[3]
32	1.7±0.2	14.1 (13.3-14.9)	

[1] Δ Body temperature = temperature 4 h after injection minus preinjection temperature.
[2] 95% confidence limits
[3] P<0.05

Conclusion : The results confirmed the hypothesis that increased environmental temperature increases the toxic effects of dinoseb in adult mice. however, the hypothesis that low environmental temperature protects adult mice from dinoseb was not supported by a shift in LD50 of dinoseb.

Reliability : (2) valid with restrictions
18.02.2003 (27)

5.2.1 SKIN IRRITATION

5.2.2 EYE IRRITATION

Species : rabbit
Concentration :
Dose : .1 ml
Exposure time :
Comment :
Number of animals : 9
Vehicle :
Result : highly irritating
Classification : irritating
Method : EPA OPP 81-4
Year : 1980
GLP : yes
Test substance : DINOSEB 5
Source: Uniroyal Chemical
Purity: 51 - 55%
Test condition : Three rabbits had their eyes irrigated 30 seconds after instillation of test material. The remaining 6 rabbits received no washout.

Result : The test material caused corneal opacity and irritation o fthe conjunctiva which persisted in some rabbits for seven days. The test material would be in EPA category I for eye irritation for rabbits.

Reliability : (1) valid without restriction
29.01.2003 (13)

Species : rabbit
Concentration :
Dose : .1 ml
Exposure time :
Comment :
Number of animals : 9
Vehicle :
Result : highly irritating
Classification : irritating
Method : EPA OPP 81-4

Year	:	1980
GLP	:	yes
Test substance	:	DINOSEB 3+1 Source: Uniroyal Chemical Purity: 51 - 55%
Test condition	:	Three rabbits had their eyes irrigated 30 seconds after instillation of test material. The remaining 6 rabbits received no washout.
Result	:	The test material was found to be extremely irritating to the rabbit eye, causing corneal opacity and irritation of the conjunctiva in all rabbits. The test material would be in EPA toxicity category I for eye irritation for rabbits.
Reliability	:	(1) valid without restriction

20.09.2002 (12)

5.4 REPEATED DOSE TOXICITY

Type	:	Subchronic
Species	:	rat
Sex	:	male/female
Strain	:	Sherman
Route of admin.	:	oral feed
Exposure period	:	153 days
Frequency of treatm.	:	daily
Post exposure period	:	
Doses	:	0, 50, 100, 150, 200, 300, 400, 500 ppm
Control group	:	
Method	:	A combination subchronic feeding and single generation reproduction protocol was used.
Year	:	1978
GLP	:	
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Purity: Technical grade (80%)
Method	:	Animals were fed with test material for 60 days, then bred and the parents and offspring continued on the study for a total exposure to the parents of 153 days. Test subjects: Age at study initiation: 35-38 days No. of animals/sex/dose: 14 rats of each sex/dose group Bioeffect paramters studied: Lethality, growth, food consumption, clinical chemistry, residue, behaviour, microsomal enzymes and pathology. Reproductive parameters: Fertility, fecundity, viability, lactation and growth.
Result	:	Body weight: Growth was depressed monotonically at 200, 150, 100 and 50 ppm Clinical biochemistry: Blood alkaline phosphatase, alanine aminotransferase, potassium and BUN were significantly increased while LDH and cholinesterase were depressed. Residue levels were dose dependent with blood > feces > urine > adipose > brain > liver. Aminopyrine N-demethylase activity was increased. Mortality and time to death: The 300, 400 and 500 ppm groups were terminated at 21 days due to mortality (14, 100 and 100%, respectively) Pathology: A significant pathological change was diffuse tubular atrophy of

the testes, particularly at 200 ppm

Organ weight changes: Organ weight (liver, spleen, heart, lung, brain) was decreased while the organ weight/body weight ratios increased

Other observations: Discrimination learning was not affected while locomotor activity was increased at 200 ppm. Fertility, fecundity, neonate survival, weight gain, viability and lactation were all depressed.

Reliability : (4) not assignable
12.03.2003 (19)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
System of testing	:	Salmonella typhimurium strains TA1535, TA1537, TA1538 TA98 and TA100
Test concentration	:	
Cycotoxic concentr.	:	no data
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: based on Ames (1971-75)
Year	:	1981
GLP	:	no
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Tested as the herbicide Premerge

Method :

Metabolic activation: S9-mix, Rat liver cells, Aroclor induced

Concentrations tested: 10 µl of neat or dilute solution added to plates. The dilution factor is unknown, but is stated to approximate to a tank mix delivering 15 gal/acre of recommended herbicide concentration.

Statistical Methods: None

Number of replicates: none

Positive Controls:
N-ethyl-N'-nitroso-N-nitrosoguanidine
9-aminoacridine
aflatoxin B1
2-aminofluorene

Negative Control: Solvent vehicle

- Solvent: Dimethylsulfoxide

Reliability : (4) Not assignable
29.01.2003 (7)

Type	:	Salmonella typhimurium reverse mutation assay
System of testing	:	Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, TA100
Test concentration	:	6 concentrations, 10 mg/plate maximum, depending on solubility and toxicity. At least 4 non-toxic concentrations tested.
Cycotoxic concentr.	:	no data
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: Ames, B.N., McCann, J. and Yamasaki, E., Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test, Mutat. Res., 31:347, 1975

Year	:	1982
GLP	:	no
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Purity: Technical grade
Method	:	Metabolic activation: rat liver S9, Arochlor 1254 induced. Statistical Methods: no data Test Design: Number of replicates: At least 2 Positive and negative controls: no data Criteria for evaluating results: A mutagenic response was defined as a dose-related increase in the number of histidine-dependant colonies per plate. A compound was considered positive when it produced a dose-related increase in the number of revertants in at least one strain for at least 3 concentration levels. A compound was considered negative when no dose-related increase in the number of revertants was observed in at least two independant experiments.
Reliability 29.01.2003	:	(4) not assignable (33)
Type	:	Escherichia coli reverse mutation assay
System of testing	:	Escherichia coli strain WP2 uvrA-
Test concentration	:	6 concentrations, 10 mg/plate maximum, depending on solubility and toxicity. At least 4 non-toxic concentrations tested.
Cycotoxic concentr.	:	no data
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: Bridges, B.A., Simple bacterial systems for detecting mutagenic agents, Lab. Pract., 21:413, 1972
Year	:	1982
GLP	:	no
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Purity: Technical grade
Method	:	Metabolic activation: rat liver S9 induced with Arochlor 1254. Statistical Methods: no data Test Design: Number of replicates: At least 2 Positive and negative controls: Positive control - 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2) without metabolic activation and 2-aminoanthracene with metabolic activation. Negative control - solvent Solvent: no data Criteria for evaluating results: A mutagenic response was defined as a dose-related increase in the number of histidine-dependant colonies per plate. A compound was considered positive when it produced a dose-related increase in the number of revertants in at least one strain for at least 3 concentration levels. A compound was considered negative when no dose-related increase in the number of revertants was observed in at least two independant experiments.
Reliability 29.01.2003	:	(4) not assignable (33)

Type	:	other: E. coli polA Differential Toxicity Assay
System of testing	:	Escherischia coli strains p3478 and W3110
Test concentration	:	Not known, but at least 2 concentrations used
Cycotoxic concentr.	:	
Metabolic activation	:	without
Result	:	positive
Method	:	other: Slater, E.E., Anderson, M.D., and Rosenkranz, H.S., Rapid detection of mutagens and carcinogens, Cancer Res., 31:970, 1971
Year	:	1982
GLP	:	no
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Purity: Technical grade
Method	:	To determine a test compound's relative toxicity to the two bacterial strains,a disk of filter paper inoculated with test substance was placed on the surfaces of two agar plates, each containing nutrient broth and one of the bacterial strains used for the assay. After the plates had been incubated for a day, the zone of inhibition of growth of bacteria by the test compound was measured. Comparision of the diameters of the zones of growth inhibition for the polA- and normal strains allowed determination of whether the test compound gave rise to DNA damage. Test Design: Number of replicates: 2 Positive control: methyl methanesulfonate Negative controls: ampicillin, kanamycin or chloramphenicol Criteria for evaluating results: A positive response was indicated by a larger zone of inhibition on the repair-deficient strain than on the normal strain, while a negative response is indicated by equal zones of growth on both plates.
Remark	:	E. coli strain p3478 is a DNA polymerase I deficient (polA-) derivative of strain W3110. The enzyme DNA polymerase I is involved in resynthesizing DNA segments that have been damaged and excised. Bacterial strains deficient in DNA polymerase I are thus more sensitive to DNA damage, and will exhibit diminished growth in the presence of DNA-damaging agents in comparison with the parent strain. Therefore, test compounds that are more toxic to p3478 than to W3100 may be assumed to react with DNA.
Reliability	:	(4) not assignable

29.01.2003 (33)

Type	:	Bacillus subtilis recombination assay
System of testing	:	B. subtilis strains M45 & H17
Test concentration	:	Not known, but at least 2 concentrations used
Cycotoxic concentr.	:	
Metabolic activation	:	without
Result	:	positive
Method	:	other: Kada, T., Mutagenicity testing of chemicals in microbial systems, in: New methods in experimental chemistry and toxicology, Coulston, F., et al, eds, International Academic Printing, Japan, 1973
Year	:	1982
GLP	:	no
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Purity: Technical grade
Method	:	To determine a test compound's relative toxicity to the two bacterial strains,a disk of filter paper inoculated with test substance was placed on

the surfaces of two agar plates, each containing nutrient broth and one of the bacterial strains used for the assay. After the plates had been incubated for a day, the zone of inhibition of growth of bacteria by the test compound was measured. Comparision of the diameters of the zones of growth inhibition for the recA- and normal strains allowed determination of whether the test compound gave rise to DNA damage.

Test Design:

Number of replicates: 2

Positive control: methyl methanesulfonate

Negative controls: ampicillin, kanamycin or chloramphenicol

Criteria for evaluating results: A positive response was indicated by a larger zone of inhibition on the repair-deficient strain than on the normal strain, while a negative response is indicated by equal zones of growth on both plates.

Remark : B. subtilis strain M45 is a recombination-deficient (recA-) derivative of strain H17. Recombination is required for repair of damaged DNA. Bacterial strains deficient in recA are thus more sensitive to DNA damage, and will exhibit diminished growth in the presence of DNA-damaging agents in comparison with the parent strain. Therefore, test compounds that are more toxic to M45 than to H17 may be assumed to react with DNA.

Reliability : (4) not assignable
29.01.2003 (33)

Type : other: S. typhimurium Differential Toxicity Assay
System of testing : Salmonella typhimurium strains SL4700, SL4525, TA1978, TA1538
Test concentration : no data
Cycotoxic concentr. : no data
Metabolic activation : without
Result : positive
Method : other: Ames, B.N., McCann, J. and Yamasaki, E., Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test, Mutat. Res., 31:347, 1975
Year : 1982
GLP : no
Test substance : 2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Purity: Technical grade

Method : Test method similar to that described in the E. coli polA differential Toxicity Assay.

Test Design:

Number of replicates: no data

Positive control: no data

Negative controls: no data

Criteria for evaluating results: A positive response was indicated by a larger zone of inhibition on the repair-deficient strain than on the normal strain, while a negative response is indicated by equal zones of growth on both plates.

Remark : S. typhimurium strain SL4700 is a recombination-deficient derivative of strain SL4525. These two strains also have an rfa- mutation that leads to a defective lipopolysaccharide coat, which makes them more permeable to larger molecules and thus more suitable for the testing of possible mutagens. TA1978 and TA1538 also contain the rfa- mutation. TA1538

lacks the uvrB gene, which is involved in the repair of DNA damage caused by exposure to uv light.

Reliability : (4) not assignable
29.01.2003 (33)

Type : Yeast gene mutation assay
System of testing : S. cerevisiae D3
Test concentration : 5 concentrations used, up to 50% toxicity
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: Brusick, D.J., and Mayer, V.W., New developments in mutagenicity screening techniques with yeast, Environ. Health Perspect., 6:83, 1973
Year : 1982
GLP : no
Test substance : 2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Purity: Technical grade

Method : The recombinogenic activity of the substance or its metabolites was determined from the number of red-pigmented colonies appearing on test plates.

Test Design:

Number of replicates: no data

Positive control: no data

Negative controls: no data

Criteria for evaluating results: A positive response in this assay was indicated by dose-related increases of more than threefold in the absolute number of mitotic recombinants per millilter and in the relative number of mitotic recombinants per 100,000 survivors. A negative response was indicated by no recombinogenic activity in any of the assays.

Remark : The yeast S. cerevisiae D3 is a diploid microorganism heterozygous for a mutation leading to a defective enzyme in the adenine-metabolizing pathway. When grown on a medium containing adenine, cells homozygous for this mutation produce a red pigment. These homozygous mutants can be generated from the heterozygotes by mitotic recombination. The frequency of this recombinational event is increased by incubation in the presence of recombinogenic agents.

Reliability : (4) not assignable
20.02.2003 (33)

Type : Unscheduled DNA synthesis
System of testing : Human lung fibroblast cells, strain WI-38
Test concentration : 5 concentrations used, do data on actual amounts
Cycotoxic concentr. : no data
Metabolic activation : with and without
Result : negative
Method : other: Simmon, V.F., In vivo and in vitro mutagenicity assays of selected pesticides, in: A rational evaluation of pesticidal vs mutagenic/carcinogenic action, Hart, R.W., et al, eds, DHEW Publication No. (NIH) 78-1306, 1978.
Year : 1982
GLP : no
Test substance : 2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Purity: Technical grade

Method : The recombinogenic activity of the substance or its metabolites was determined from the number of red-pigmented colonies appearing on test plates.

Test Design:

Number of replicates: 6

Positive controls: 4-nitroquinoline-N-oxide (-S9), dimethylnitrosamine (+S9)

Negative controls: DMSO in culture medium

Criteria for evaluating results: The test material was considered positive if there was a significant concentration-related increase in thymidine incorporation in test material cells compared to the negative controls, negative if no significant increase in thymidine incorporation was observed.

Reliability : (4) not assignable
29.01.2003 (33)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Drosophila SLRL test
Species : Drosophila melanogaster
Sex : male
Strain :
Route of admin. : oral feed
Exposure period : no data
Doses : no data
Result : negative
Method : other: Waters, M.D., et al, An overview of short-term tests for the mutagenic and carcinogenic potential of pesticides, J. Environ. Sci. Health, B15:867, 1980

Wurgler, F.E., et al, Drosphila as assay system for detecting genetic changes, in: Handbookof Mutagenicity Test Procedures, Kilbey, B.J., et al, eds, Elsevier/North Holland Biomedical Press, Amsterdam, 1977
Year : 1982
GLP : no
Test substance : 2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Purity: Technical grade

Method : Males that carry the genes BAR (B) and yellow (y) on the X chromosome plus two minute secondary translocations of the X chromosome, bearing the wild-type allele (y+), one on each arm of the Y chromosome, were exposed to the test chemical. The exposed males were crossed to females carrying the Inscy X chromosome in homozygous condition; this doubly inverted X is marked with y and scute (sc). The male and female progeny of this mating (F1) were in turn mated with each other and the resulting progeny (F2) were examined. Absence of the treated male phenotype in the F2 generation was considered evidence of recessive lethal mutation.

Cultures of the F2 generation were examined under a dissecting microscope approximately 2 weeks after initiation of the brood. If at least 2 Bar-eyed males were present, the culture was scored as non-lethal. It there were at least 20 progeny and no Bar-eyed males, the culture was retested for confirmation of lethality, as were cultures with less than 20 progeny and a low ratio of Bar-eyed males to Bar-eyed females.

In the retest, 3 F2 females of the yellow-Bar phenotype were mated with their F2 male siblings, and their progeny (F3) were examined. If no Bar-eyed males occurred among more than 20 offspring, the culture was scored as lethal. If some F2 females tested lethal and some nonlethal, their gonads were mosaic for sex-linked recessive lethal mutation and this

group was also scored as lethal. If less than 20 progeny emerged from all the F3 cultures, this test was eliminated from final scoring calculations.

On completion of the scoring of all the test results, the tabulated results were subjected to statistical analysis to determine an overall mutation frequency.

A compound that induced an increase in the background mutation rate of at least 0.2% at the 95% statistical confidence level was considered to elicit a positive response.

Compounds that were tested with a sample population sufficient to permit detection at the 95% statistical confidence level but did not elicit a 0.2% increase in mutation rate over the background level were classified as negative.

Reliability : (4) not assignable
07.11.2002 (33)

5.8.1 TOXICITY TO FERTILITY

Type	:	One generation study
Species	:	rat
Sex	:	male
Strain	:	Sherman
Route of admin.	:	oral feed
Exposure period	:	Up to 77 days
Frequency of treatm.	:	daily ad libitum
Premating exposure period		
Male	:	77 days
Female	:	
Duration of test	:	
No. of generation studies	:	
Doses	:	
Control group	:	
Method	:	
Year	:	1982
GLP	:	
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Purity: 97.3% Source: Dow Chemical Company Lot No.: AGR133942

Method : Study Design:

Sex: Male animals were fed with the test substance.
Age: Adult, 99-115 days of age

Doses/No. of animals per dose: 0 ppm (36 males), 75, 150, 225 ppm (20 males/group), 300 ppm (36 males). Pair weight control group (0 ppm, 20 males)

Rats in the pair weight (PW) group were matched with a partner of similar weight in the 300 ppm group and their food restricted so that the rate of weight loss was similar in the 2 groups.

Four rats in groups fed 0 or 300 ppm were sacrificed for terminal studies after 10, 20, 30 and 50 days of treatment. One half the surviving rats in each of the 6 groups were sacrificed for terminal studies during the eleventh week of treatment (71-77 days). The remaining rats were used

for reproductive studies and subsequent return phase terminal studies.

Reproduction studies:

After 77 days of feeding all animals scheduled for breeding were fed untreated pellet ration ad libitum. The males were pair bred with each of 2 untreated virgin females during the 2-week period following the end of treatment. The presence of a copulatory plug was considered evidence of insemination. Counting the day of insemination as Day 0, females were killed on Day 20 and implants, fetal viability and fetal weights recorded. The same males were again bred 104-112 days after withdrawal of treatment.

Terminal studies:

Sperm counts were measured and sperm cells examined for abnormalities.

Statistical methods:

Body weights, organ weights, sperm counts and body temperature were subjected to one-way or two-way analysis of variance. Sperm counts of macerated tissue were adjusted for the tissue weight and organ weights were adjusted for terminal body weight by analysis of covariance. When analysis of variance indicated significant differences between groups, means or adjusted means were compared by Duncan's multiple range test. Sperm morphology profiles were established with cluster analysis and group comparisons were made with Fisher's exact test. The Mann-Whitney U test was used to compare resorptions.

Result : Actual Dose received: The dosage of dinoseb over the treatment period averaged 3.8, 9.1, 15.6 and 22.2 mg/kg/day for groups fed 75, 150, 225 and 300 ppm, respectively.

Body weight: Male rats fed the 2 highest concentrations of dinoseb lost weight rapidly. The average weight loss after 70 days was 19 and 38% for groups fed 225 and 300 ppm, respectively. The body weights of rats fed 150 ppm stayed relatively constant. Animals fed 75 ppm had weight gains similar to that of the controls. Upon cessation of treatment rats rapidly regained weight. After the 16-week recovery period mean body weights of the PW and 150 ppm groups had reached the levels of the control and the weights of rats which had been fed 225 ppm were only slightly less than controls. The rats fed 300 ppm, although exhibiting substantial gain during the recovery period, did not attain their original weights.

Clinical signs: Body temperatures were elevated in the PW and 300 ppm groups ($p<0.05$), compared to the control group, after 9 days and in the 225 ppm group after 16 days. Temperatures of rats fed 75 and 150 ppm were similar to controls.

Signs of toxicity were observed in groups fed 225 and 300 ppm. The animals in these groups appeared weak and often assumed atypical sprawling postures. Irregular and rapid breathing was common and severely affected animals sometimes salivated profusely. All rats fed 300 ppm and several fed 225 ppm developed distinct wheezing which abated toward the end of treatment and disappeared in all but one rat during the post treatment observation period. Yellow staining of the fur from contact with the dinoseb diet was evident. Definite signs of toxicity were not observed in groups fed 75 or 150 ppm. The rats in the PW group were more irritable than controls but, except for emaciation, appeared normal.

Mortality: Nine males fed 300 ppm died between 36-70 days of treatment and one died on the third day of withdrawal. One rat fed 225 ppm and one

control died after 57 and 59 days of treatment, respectively.

Reproductive performance: None of 5 males fed 300 ppm and only one of 10 animals fed 225 ppm produced litters in the 14-day period after discontinuation of treatment. This infertility was not accompanied by loss of libido, since the incidence of copulations in rats fed 225 ppm was similar to controls. Some evidence of coitus was also observed at 300 ppm. In the PW group, 9/10 males produced litters. The fertility index was somewhat lower than controls in the PW, 75 and 150 ppm groups, although the number of fertile males, viable litter size, resorptions, and fetal weights were comparable. Mating of the same males 15 weeks after discontinuation of treatment produced essesntially the same results, except possibly in the PW group. Similar to the first mating, 0/5 and 2/10 males which had been fed 300 ppm and 225 ppm, respectively, produced litters. No overt effect was observed in rats which had been fed 75 or 150 ppm, however only 4/10 males in the PW group sired litters.

Organ weights and gross pathology: In rats fed 300 ppm dinoseb, which were killed after 20, 30 and 50 days of treatment, unadjusted weights of the epididymides, seminal vesicles and prostate were generall less ($p<0.05$) than those in control groups. The weights of the testes were decreased ($p<0.05$) only at 50 days. After adjustment for terminal body weight, weights of testes and epidiymides did not differ from the controls through 50 days of treatment. Inconsistent decreases were seen in the weights of the seminal vesicles at 20 and 50 days, and in the prostate at 20 days. The testes were flaccid in 1/4, 2/4 and 4/4 dinoseb treated rats killed at 20, 30 and 50 days, respectively. The gross appearance and weights of organs of rats killed after 10 days were similar in control and dinoseb treated groups. In animals killed during the eleventh week of treatment, the testes of rats fed 225 or 300 ppm were small and flaccid. The seminiferous tissue had a liquid consistency. The epidiymides in these two groups were also very small and the surface, especially of the caudal portions, appeared greyish. Epididymides in the PW group were smaller than in controls but appeared normal in other respects. The prostate and seminal vesicles in the PW, 225 and 300 ppm groups were quite small, but other abnormalities were not evident. Weights of the reproductive organs organs of rats killed during the eleventh week of treatment were measured. Comparisons of adjusted mean weights of the testis and epididymis of rats fed 225 and 300 ppm and the seminal vesicle weights of rats fed 300 ppm were decreased when compared to control and PW groups. The appearance of organs in rats fed 75 and 150 ppm were similar to controls and organ weights were similar or greater than controls. There was no difference in adjusted organ weights of the PW and control group. In rats killed 16 week safter withdrawal of treatment, the appearance of the tested and epididymides in rats that had been fed 225 and 300 ppm were essentially as described for these groups at the end of treatment. Exceptions were two rats that had been fed 225 ppm in which these organs appeared normal. Absolute weights of the testes and epididymides in these groups were slightly greater than in rats killed at the end of treatment, but adjusted weights were still markedly decreased compared to the control group. In rats fed 300 ppm, adjusted weights of the prostate were less than in controls. Both unadjusted and adjusted organ weights in the PW group were comparable to the control group.

Sperm studies: By 30 days of treatment, epididymal sperm counts of rats fed 300 ppm were decreased ($p<0.05$), the values being 30 and 17% of control for epididymal fluid and unadjusted macerated tissue counts, respectively. In rats killed at 50 days, these values declined further to 6 and 8% of the control values. In rats killed during the eleventh week of treatment, the small size and decreased fluid content of the epididymides in rats fed 300 ppm made samples of fluid difficult to collect. After the 16 week recovery period, sample collection was also encountered in those

which had been fed 225 ppm. The epididymal fluid in these two groups was usually clear and colorless, whereas in other groups, the fluid was usually clear and colorless, whereas in other groups the fluid had a creamy opaque appearance. In rats killed during the eleventh week of treatment, both epididymal fluid and macerated tissue sperm counts indicated severe oligospermia in rats fed 225 and 300 ppm. Azoospermia was observed in only a few animals. Reduced sperm counts were also observed in rats fed 150 ppm, the values being 83 and 54% of control for epididymal fluid and unadjusted macerated tissue counts, respectively. Epididymal fluid counts in the PW and 75 ppm groups were similar to controls, but the mean macerated tissue count in the PW group was only 46% of the control value. Adjustment for tissue weight indicated sperm counts of macerated tissue were related to tissue weight in the PW group, but the adjusted counts in rats fed 150 ppm were only 60% of the controls. After the 16 week recovery period oligospermia was observed only in rats which had been fed 225 or 300 ppm.

Motile spermatozoa were observed in epididymal fluid suspensions in all rats in the control, PW and 75 ppm groups. Non-motility was observed in only one rat fed 150 ppm. This particular animal had very low sperm counts and failed to produce a litter in 3 matings. No motile spermatozoa were observed in rats fed 225 ppm killed at the end of treatment, but 2/10 rats killed after 16 weeks of withdrawal had motile cells. Motile spermatozoa were observed in only 2/4 rats fed 300 ppm which were killed on day 20 of treatment. No motile spermatozoa were seen in any of the subsequent sacrifices of rats fed 300 ppm including those killed after the 16 week recovery period.

Only 10% of the epididymal sperm cells were classified as normal in the rats fed 300 ppm which were killed on day 20 of treatment. Atypical forms at this time were primarily isolated heads (complete cleavage of the head and tail) and incipient separations. In the latter category, these was a separation of the sheath which presented a frayed appearance invariably occurring at the neck or the midpiece-mainpiece juncture. Also in this category, many cells showed only a distinct degeneration at the midpiece-mainpiece annulus. In rats killed at 30 and 50 days, many amorphous bizarre forms were also observed. These included cells with multiple nuclear fragments, cells which exhibited gross distortion of the size and shape of the cytoplasm and multitailed forms which had the appearance of fused cells. Some cells showed all of these abnormalities. Severe oligospermia prevented differential classification in the subsequent sacrifices of animals fed 300 ppm. Marked oligospermia allowed classification in only three of the rats fed 225 ppm which were killed during the eleventh week of treatment. An increased incidence of bizarre forms was also observed in this group. Many spermatozoa in rats fed 150 ppm were also atypical, only 45% being classified as normal. The major abnormalities in this group were decapitate cells and incipient separations, with all rats showing abnormal profiles. Two of 10 rats in the PW group had abnormal profiles. The incidence of atypical forms in rats fed 75 ppm was similar to the control groups. The sperm abnormalities observed in rats fed 150 ppm appeared to be reversible; however, of 5 rats fed 225 ppm that were classified at the end of the 16 week recovery period, 3 had abnormal profiles. The abnormalities were mainly decapitate cells and incipient separations.

Microscopic Findings: Histopathologic changes in the testes and caput portion of the epididymides involved primarily the germinal elements. No obvious effects were detected in the interstitial cells of the testes or in the epithelial cells of the epididymides. Microscopically, the testes of the rats fed 300 ppm dinoseb for 10 days exhibited no abnormalities and resembled those of the control rats. however, in one of the four rats some abnormal forms of spermatozoa were apparent in the epididymides. These forms

were rarely seen in controls. Slight changes were noted in two rats killed after 20 days. A few testicular tubules contained abnormally large spermatids and several abnormal forms of spermatozoa. abnormal spermatozoa and a greater than normal number of desquamated spermatogenic cells were present in the epididymides. A greater degree of change was observed in the other rats killed at 20 days and in two rats killed at 30 days, in which several to many testicular tubules were affected. Multinucleated spermatogenic cells were seen in the tubules. Also noted were focal areas with some loss of spermatocytes and spermatogonia, sometimes associated with the presence of occasional eosinophilic staining necrotic spermatogenic cells. The number of epididymal spermatozoa was considerably decreased and some of these appeared abnormal. In the other two rats killed at 30 days these changes were even more pronounced and some testicular tubules contained no spermatogenic cells and were lined by Sertoli cells only. Intact spermatogenic cells could be seen in only a few tubules. Epididymal spermatozoa were few in one rat and not seen in the other. The most severe change was observed in rats fed 300 ppm and killed at 50 days, and in those fed 225 or 300 ppm and killed during the eleventh week, in which almost every testicular tubule was involved. Necrotic spermatogenic cells and cellular debris were seen in some (often many) tubules. Often, tubules were devoid of spermatogenic cells and only Sertoli cells remained. Only an occasional tubule could be identified with some intact spermatogenic cells in them. Spermatozoa were not seen in sections of the epididymides. Minimal change was seen in four rats fed 150 ppm which were killed during the eleventh week. This consisted of occasional multinucleated spermatogenic cells and abnormal forms of spermatozoa in the testes and/or epididymides. In one of the rats, in which there were a few more multinucleated cells, a moderate decrease in the number of epididymal spermatozoa was also noted. Multinucleated spermatogenic cells were not seen in the control, PW or 75 ppm groups. In one PW rat sacrificed during the eleventh week of food restriction, both testes weresmaller than those of the other rats in this group and the tubules were lined by intact spermatogenic cells without necrosis, but contained relatively few mature spermatids or spermatozoa. Only occasional spermatozoa were observed in the epididymides.

In rats sacrificed 16 weeks after withdrawal of treatment, the severe changes in rats fed 300 ppm persisted and were similar to those described for this group at the end of treatment. There was no evidence of regeneration of the germinal epithelium in the testes. Similarly, in 8 out of 10 rats fed 225 ppm, the changes were as described at the end of treatment. In another rat of this group, the histologic appearance was comparable to that in the control rats. The tenth animal in this group had partial involvement only in one fourth of one testis. The affected tubules lacked spermatogenic cells, were lined by Sertoli cells only, and showed no evidence of necrotic spermatogenic cells. A moderate decrease in the number of spermatozoa was noted in the corresponding epididymis. It was not clear whether the lesion in this animal was related to treatment or was only an incidental partial atrophy, since the lesions attributable to dinoseb in most of the rats in this group were prominent and bilateral. Changes were seen in only one rat of the 150 ppm group killed 16 weeks post-treatment. The tubules in both testes were devoid of spermatogenic cells and were lined by Sertoli cells only. Necrotic spermatogenic cells and cellular debris were not evident. Only a few tubules contained intact spermatogenic cells and showed little evidence of spermatozoa formation. A few spermatozoa could be identified in the epididymides. although this bilateral lesion might have resulted from the dinoseb treatment, it was seen in only one animal of this group. similar loss of spermatogenic cells without necrosis was also observed in all tubules of one testis of a single rat in the PW group. An incidental testicular change was seen in several control rats, consisting of single tubules or occasionally small groups of tubules, usually in the periphery of one or both testes, that were devoid of all

spermatogenic cells and contained only Sertoli cells. It was interpreted as spontaneous focal tubular atrophy. When similar focal change was observed in several rats in the treated and PW groups in which the testes were otherwise normal, it was also considered to be incidental since its incidence was not significantly different in any of the groups. Rats fed 300 ppm dinoseb which were killed during the eleventh week of treatment and those of the PW group which were killed during the eleventh week of food restriction, showed microscopic evidence of atrophy in the accessory reproductive organs, particularly the seminal vesicles and coagulating glands. No such atrophy was seen in any groups sacrificed 16 weeks after resumption of the normal diet. No significant changes were identified in the lungs and bronchi that would account for the wheezing noted in rats fed 225 and 300 ppm.

Reliability : (1) valid without restriction
28.02.2003 (24)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	mouse
Sex	:	female
Strain	:	Swiss Webster
Route of admin.	:	other: ip or sc injection or oral intubation
Exposure period	:	Days 8-16, 10-12 or 14-16 of gestation
Frequency of treatm.	:	daily
Duration of test	:	Foetal examination on day 19 of gestation
Doses	:	
Control group	:	
Method	:	other:
Year	:	1973
GLP	:	no
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Source: Dow Chemical Company Lot No.: 7200206

Method : METHOD

Method/guideline: The test material was administered daily, ip, sc or by oral intubation, to groups of pregnant mice either throughout organogenesis (days 8-16 of gestation), during early organogenesis (days 10-12), or during late organogenesis (days 14-16). The dosage levels were selected as those expected to produce (1) maternal toxicity, (2) no maternal toxicity and (3) no embryotoxic or teratogenic effect. Foetuses were removed by caesarian section for examination on day 19 of gestation.

Type: One generation

Doses/concentration levels:

IP Injection:

Days 10-12: 0, 10, 12.5, 15.8, 17.7, 18.8, 20 mg/kg/day
Days 14-16: 0, 12.5, 17.7 mg/kg/day
Days 8-16: 5 mg/kg/day

SC Injection:

Days 10-12: 0, 10, 17.7 mg/kg/day
Days 14-16: 0, 10, 17.7 mg/kg/day
Days 8-16: 0, 10, 17.7 mg/kg/day

Oral intubation:

Days 10-12: 0, 20, 32, 50 mg/kg/day
Days 14-16: 20, 32 mg/kg/day
Days 8-16: 20, 32 mg/kg/day

Statistical methods: Statistical analysis of measured parameters was by analysis of variance (completely random design). Treatment differences were detected by the least significant difference test ($p<0.05$): Steel, R.G.D. & Torrie, H.H., Principles and Procedures of Statistics, McGraw-Hill Book Company, Inc., New York, 1960.

Remarks:

Test animals: See Table 1 for No. of animals used per dose.

Mating procedures: Pregnant mice were obtained by daily pen breeding from 8 to 9 a.m. (one male/five females). Mice with vaginal plugs were identified as being at day 1 of gestation and were isolated into treatment groups.

Standardization of litters: No

Parameters assessed during study:

Foetuses were weighed after caesarian section and crown-rump distance measured.

Organs examined at necropsy (macroscopic and microscopic): Foetuses were examined for external anomalies, soft tissue anomalies and skeletal anomalies.

Result : RESULTS

NOEL for teratogenicity and embryotoxicity throughout organogenesis (days 8-16):

IP: 5 mg/kg/day
SC: 10 mg/kg/day
Oral: 20 mg/kg/day.

Parental data and F1 as appropriate (toxic response/effects with NOAEL value):

IP administration of test material:

In ip doses of 17.7-20.0 mg/kg/day, the test material produced signs of toxicity (hyperthermia) in the dams and some maternal deaths (Table 1). Surviving dams bore litters that were smaller in number and size than those of controls regardless of the gestational stage at the time of administration (Table 2). Doses of the test material that were sub-toxic to the dams (10-15.8 mg/kg/day) did not affect the resorption rate or foetal size when given during early organogenesis (days 10-12) but were foetotoxic when given later (days 14-16). Test material given throughout organogenesis (days 8-16) in ip doses of 5 mg/kg/day had no adverse effect on embryonic or foetal growth and development (Table 2).

Statistically significant incidences of gross, soft-tissue and skeletal anomalies were induced in the offspring of dams given the test material ip during early stage organogenesis (Table 3). It should be noted that the effects were considered to be significant relatively frequently in the group given 17.7 mg/kg/day in which only 1/14 dams died, but were rarely significant in the group given 18.8 mg/kg/day in which 5/11 died. No gross,

soft-tissue or skeletal anomalies were detected in foetuses from dams given 5 mg/kg/day throughout gestation (days 8-16), nor in foetuses from dams given any dose level on gestational days 14-16. On the basis of these parameters, the 5 mg/kg/day ip dose of test material was considered to have produced no effect.

SC administration of test material:

Test material administered sc in doses of 17.7 mg/kg/day produced overt toxicity similar to that observed with ip administration. At this dosage, the test material significantly affected foetal survival and size, but only when administered during late organogenesis (days 14-16) or throughout organogenesis, days 8-16 (Table 4).

Administered sc, the test material only produced statistically significant gross or soft-tissue anomalies when given in doses of 17.7 mg/kg/day on days 14-16. This treatment resulted in an incidence of cleft palate of 10.6±6.8%. The actual significance of cleft-palate induction by the test material is uncertain because this anomaly did not have a statistical significance in any ip-treated group or in the sc-treated group given 17.7 mg/kg/day throughout days 8-16. Skeletal examination showed an increased incidence of certain anomalies in the offspring of dams treated with 17.7 mg/kg/day on gestational days 10-12 and 8-16 (Table 5), a finding which indicated foetotoxicity as well as maternal toxicity. Examination of foetuses from the dams given 10 mg/kg/day revealed no effect on the parameters studied (Table 5).

Oral administration of test material:

Test material given orally in the doses used in the ip and the sc studies produced no overt maternal toxicity, so higher dose levels were selected. Doses of 50 mg/kg/day given on days 10-12 of gestation approximated to an LD75 (Table 6) for the maternal animal but had no effect on foetal survival or size. Likewise, doses of 20 or 32 mg/kg/day administered orally during early or late organogenesis or throughout this whole period caused maternal toxicity but did not significantly affect foetal survival or size, except in mice given 32 mg/kg/day on days 8-16, in which the foetal crown-rump distance was reduced to 2.4 cm compared with 2.6 cm in controls.

Oral administration of test material produced no statistically significant gross or soft-tissue anomalies at any time. Statistically significant incidences of skeletal anomalies were observed in some of the groups (Table 7) but only at doses that were lethal to some dams. High incidences of the same anomalies in controls ins pecific comparisons precludes any assessment of the actual significance of these findings.

At the lowest treatment level (20mg/kg/day), no pregnant mice died when treated orally on days 10-12 or days 14-16. However, 1/8 mice died when treatment was given throughout the entire 9 day period (days 8-16). Even so, the foetuses from dams treated at this level showed no effects of practical significance.

Table 1: Mortality of dams during treatment with dinoseb

Dose level (mg/kg/day)	Mortality of dams (No. Dead/No. Treated) following treatment by		
	IP injection	SC injection	Oral intubation
	On days 10-12		
0	0/8	0/7	0/6
10	0/11	0/7	-
12.5	0/7	-	-
15.8	0/7	-	-
17.7	1/14	0/7	-
18.8	5/11	-	-

20	4/4	-	0/8
32	-	-	0/8
50	-	-	6/8
	On days 14-16		
0	0/7	0/8	-
10	-	0/8	-
12.5	0/7	-	-
17.7	4/12	1/8	-
20	-	-	0/14
32	-	-	2/11
	On days 8-16		
0	-	0/5	-
5	0/7	-	-
10	-	0/8	-
17.7	-	0/8	-
20	-	-	1/8
32	-	-	2/9

Table 2: Resorption rate and foetal size in pregnant mice given dinoseb by ip injection during various stages of organogenesis

Dose level (mg/kg/day)	No. of pregnant mice		No. of implantations †	No. of foetuses †	Resorptions † (%)	Foetal body weight † (g)	Foetal crown-rump length † (cm)
	Treated	Surviving					
			Days 10-12‡				
0	8	8	13±1	13±1	3.5±1.3	1.357±0.036	2.6±0
10	11	11	13±1	12±1	11.3±4.0	1.334±0.026	2.6±0
12.5	7	7	13±1	11±1	8.1±2.4	1.250±0.047	2.5±0
15.8	7	7	12±1	11±1	8.1±2.7	1.283±0.028	2.5±0
17.7	14	13	12±0	8±1*	33.9±7.4*	1.060±0.036*	2.5±0
18.8	11	6	13±1	12±1	4.5±2.1	1.138±0.024*	2.3±0*
20.0	4	0	-	-	-	-	-
			Days 14-16‡				
0	7	7	13±1	12±1	8.7±4.5	1.355±0.030	2.5±0
12.5	7	7	13±1	10±2	36.7±11.1*	1.169±0.036*	2.5±1
17.7	12	8	14±1	4±2*	71.2±11.6*	1.101±0.040*	2.4±0*
			Days 8-16‡				
5	7	7	12±1	11±1	8.8±3.2	1.270±0.036	2.6±0

† Mean response/litter±SEM

‡ Days of gestation on which treatment was given

Values marked with an asterisk differ significantly from those of the controls: *P<0.05

Table 3: Gross, soft-tissue and skeletal anomalies in offspring of pregnant mice given dinoseb by ip injection during early organogenesis (days 10-12)

	Incidence of anomalies following treatment at dose levels (mg/kg/day) of					
	0	10.0	12.5	15.8	17.7	18.8
Anomalies No. of litters examined..	8	11	7	7	13 †	6
Gross						
Oligodactyly	0	0	1.3±1.3	0	35.9±9.8*	15.0±13.1
Imperforate anus	0	0	0	2.9±2.9	19.2±8.7*	6.7±6.7
Acaudia	0	0	0	2.9±2.9	17.5±7.7*	1.7±1.7
Microcaudia	0	0	0	0	25.3±8.0*	7.8±6.5
Brachygnathia	0	0	0	0	2.5±2.5	0
Amelia	0	0	0	0	16.3±8.7*	0
Micromelia	0	0	0	0	5.5±3.9	8.3±8.3
Open eyes	0	3.6±3.0	1.1±1.1	8.3±5.9	0	0
Soft-tissue						
Internal hydrocephalus	14.6±8.4	92.0±3.0*	97.1±2.9*	55.9±11.5*	76.2±8.6*	20.5±5.5
Hydronephrosis	5.4±5.4	15.6±4.5	23.4±6.3	18.9±10.2	31.6±8.1*	18.5±5.7
Cleft palate	1.8±1.8	1.3±1.3	0	0	0	0
Enlarged bladder	3.6±2.4	0	0	9.4±4.6	1.4±1.4	8.2±5.3
Adrenal agenesis	0	0	0	0	16.2±9.9	10.0±10.0
Skeletal						
Ribs: supernumerary	27.2±12.2	13.1±6.8	23.4±13.6	20.7±6.1	26.0±8.9	24.7±5.9
fused	0	0	22.1±9.4	14.3±14.3	54.5±11.6*	37.2±13.9*
absent	0	0	0	0	12.5±7.7*	0
Sternebrae: fused	0	0	0	0	15.6±7.8*	0
absent or not ossified	6.6±4.3	11.2±3.6	19.7±7.2	34.0±13.9	56.5±9.8*	25.8±13.6
Vertebrae: fused	0	0	4.4±2.9	2.0±2.0	76.2±8.1*	37.2±13.9*
not ossified	0	0	0	0	19.5±8.8*	0
absent	0	0	0	7.1±7.1	30.8±10.5	0
Long bones absent or not ossified	0	0	4.8±3.1	9.1±7.1	41.3±10.7*	13.3±9.9

† Only 12 litters examined for soft-tissue anomalies

Values are the mean percentage responses/litter±SEM and those marked with asterisks differ significantly from those of controls: *P<0.05

Table 4: Resorption rate and foetal size in pregnant mice given dinoseb by sc injection during various stages of organogenesis

Dose level (mg/kg/day)	No. of pregnant mice		No. of implantations †	No. of foetuses †	Resorptions † (%)	Foetal body weight † (g)	Foetal crown-rump length † (cm)
	Treated	Surviving					
			Days 10-12‡				
0	7	7	11±1	11±1	4.4±2.2	1.339±0.032	2.6±0
10	7	7	14±1	13±1	5.2±3.0	1.348±0.033	2.6±0
17.7	7	7	13±1	12±1	10.0±5.4	1.224±0.040	2.6±0
			Days 14-16‡				
0	8	8	13±0	12±0	5.6±2.4	1.373±0.030	2.6±0
10	8	8	14±1	12±1	14.0±6.4	1.309±0.041	2.6±0
17.7	8	7	14±1	7±2*	46.4±15.3*	1.209±0.065*	2.5±0*
			Days 8-16‡				
0	5	5	13±1	12±1	6.4±1.7	1.304±0.042	2.6±0
10	8	8	14±1	13±0	7.2±2.8	1.315±0.026	2.6±0

17.7	8	8	12±1	10±1	14.0±7.4	1.083±0.039*	2.4±0*

† Mean response/litter±SEM

‡ Days of gestation on which treatment was given

Values marked with an asterisk differ significantly from those of the controls: *P<0.05

Table 5: Skeletal anomalies in offspring of pregnant mice given dinoseb by sc injection during early organogenesis (days 10-12)

Dose level (mg/kg/day)	No. of litters examined	Incidence of skeletal anomalies (mean percentage response/litter±SEM)						
		Ribs		Sternebrae		Vertebrae		
		Supernumerary	Fused	Absent or not ossified	Fused	Supernumerary	Fused	Absent
				Days 10-12 †				
0	7	26.1±10.2	0	2.4±2.4	0	0	0	0
10	7	14.3±11.7	4.7±4.7	22.8±8.4	0	0	0	0
17.7	7	39.0±8.8	33.0±10.7*	35.6±15.0*	2.4±2.4	6.0±4.0	50.3±9.8*	2.9±2.9
				Days 14-16 †				
0	8	20.5±10.8	0	16.0±5.2	0	0	0	0
10	8	7.2±4.8	0	11.6±6.0	0	1.6±1.6	2.5±2.5	0
17.7	5	19.4±9.8	0	31.2±9.5	0	0	0	0
				Days 8-16 †				
0	5	28.0±9.9	0	2.8±2.8	0	0	0	0
10	8	44.1±12.0	0	16.4±5.0	0	8.0±6.2	0	0
17.7	8	88.4±6.6*	8.8±4.4	39.5±6.6*	6.6±4.3	26.2±11.8*	7.1±5.4	0

† Days of gestation on which treatment was given

Values marked with an asterisk differ significantly from those of controls: *P<0.05

Table 6: Resorption rate and foetal size in pregnant mice given dinoseb by oral intubation during various stages of organogenesis

Dose level (mg/kg/day)	No. of pregnant mice		No. of implantations †	No. of foetuses †	Resorptions † (%)	Foetal body weight † (g)	Foetal crown-rump length † (cm)
	Treated	Surviving					
				Days 10-12‡			
0	6	6	10±2	9±2	4.7±3.3	1.315±0.042	2.6±0
20	8	8	11±1	11±1	3.5±1.8	1.296±0.030	2.6±0
32	8	8	14±1	13±1	4.4±1.3	1.326±0.026	2.6±0
50	8	2	13±0	12±0	4.0±4.0	1.268±0.039	2.5±0
				Days 14-16‡			
20	14	14	13±0	11±0	8.0±2.7	1.261±0.022	2.5±0
32	11	9	12±1	11±1	7.4±2.8	1.214±0.033	2.5±0
				Days 8-16‡			
20	8	7	14±1	14±1	3.1±2.1	1.296±0.033	2.6±0
32	9	7	12±1	11±1	9.0±2.2	1.202±0.057	2.4±0*

† Mean response/litter±SEM

‡ Days of gestation on which treatment was given

Values marked with an asterisk differ significantly from those of the controls: *P<0.05

Table 7: Skeletal anomalies in offspring of pregnant mice given dinoseb by oral intubation during early organogenesis (days 10-12)

Dose level (mg/kg/day)	No. of litters examined	Incidence of skeletal anomalies (mean percentage response/litter±SEM)		
		Ribs	Sternebrae	Vertebrae
		Supernumerary	Absent or not ossified	Supernumerary
		Days 10-12 †		
0	6	19.0±16.4	16.7±16.7	0
20	8	28.8±11.8	16.5±7.1	2.5±2.5
32	8	6.2±4.4	3.5±3.5	0
50	2	7.0±7.0	7.0±7.0	0
		Days 14-16 †		
20	14	7.4±3.8	13.2±5.5	0
32	9	26.9±9.0	46.8±10.2*	0
		Days 8-16 †		
20	7	65.0±10.3*	15.9±4.6	5.6±2.7
32	7	92.1±5.6*	21.1±11.6	43.0±13.7*

† Days of gestation on which treatment was given

Values marked with an asterisk differ significantly from those of controls: *P<0.05

Conclusion : The test material was concluded to have a low potential for the induction of terata in mice. Effects were dependant on the route of administration and

the dosage, and the greatest number of statistically significant anomalies was induced when the test material was administered ip. Administration by the sc or oral route had less effect on embryonic and foetal development. Doses required to induce these anomalies caused toxic effects in the dams and were in the lethal range.

Reliability : (2) valid with restrictions
29.01.2003 (18)

Species	:	mouse
Sex	:	female
Strain	:	Swiss Webster
Route of admin.	:	i.p.
Exposure period	:	Days 10, 11, and or 12 of gestation
Frequency of treatm.	:	daily
Duration of test	:	Foetal examination on day 19 of gestation
Doses	:	
Control group	:	
Method	:	other:
Year	:	1975
GLP	:	no
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Source: Dow Chemical Company Lot No.: 7200206

Method : To study the effects of food deprivation on test material induced teratogenicity, three groups of pregnant mice were deprived of food for 0, 24 or 48 hours beginning the 9th day of gestation; subgroups of these mice were treated with test material on days 10-12 of gestation. In a second experiment two groups of pregnant mice were given single injections of test material on day 11 or day 12 of gestation. For approximately half the mice in each group, treatment with test material was preceeded by 50 mg/kg phenobarbital pretreatment twice daily for 3 days; the remaining mice were not pretreated. Two other groups received test material on day 12 of gestation 1 hour after treatment with 32.0 mg/kg SKF-525A. A third group was the untreated control. Foetuses were removed by caesarian section for examination on day 19 of gestation.

Type: One generation

Doses/concentration levels:

Experiment 1: 0, 14.1, 15.8 mg/kg/day on days 10-12 of gestation

Experiment 2: 17.7 mg/kg on day 11, or 18.8 mg/kg on day 12 of gestation.

Experiment 3: 15.8, 17.7 mg/kg on day 12 of gestation, 1 hr after treatment with SKF-525A.

Statistical methods: Analysis of variance using between-group comparisons by the least significant difference test ($P<0.05$): Steel, R.G.D. & Torrie, H.H., Principles and Procedures of Statistics, McGraw-Hill Book Company, Inc., New York, 1960.

Remarks:

Test animals: See Tables 1 & 2 for No. of animals used per dose.

Mating procedures: Pregnant mice were obtained by daily pen breeding from 8 to 9 a.m. (one male/five females). Mice with vaginal plugs were identified as being at day 1 of gestation and were isolated into treatment groups.

Standardization of litters: No

Parameters assessed during study:

Foetuses were weighed after caesarian section and crown-rump distance measured.

Organs examined at necropsy (macroscopic and microscopic): Foetuses were examined for external anomalies, soft tissue anomalies and skeletal anomalies.

Result : RESULTS

Parental data and F1 as appropriate (toxic response/effects with NOAEL value):

In general, food deprivation for 24 hours increased the incidence of anomalies obtained with a teratogenic dose (15.8 mg/kg/day) of test material but not the incidence obtained with a non-teratogenic dose (14.1 mg/kg/day). Food deprivation for 48 hours generally failed to alter the incidence of test material-induced terata but enhanced the fetal body weight reduction observed with a 14.1 mg/kg/day dose (Table 1).

A dose of 15.8 mg/kg/day resulted in a low but statistically significant incidence of external anomalies when expressed as a reduction in the percentage of normal foetuses (Table 1). The incidence of typical test material-induced anomalies - such as amelia or micromelia, ectrodactyly or brachydactyly, club foot, and acaudia or microcaudia- was increased 2-5 times by 24-hr food deprivation but was not affected by 48-hr food deprivation. The percentage of normal foetuses in soft tissue examinations was decreased by a dose of 15.8 mg/kg/day or 48-hr food deprivation (Table 1). Hydronephrosis was the most frequent soft tissue anomaly; however there was no indication that a dose of 15.8 mg/kg/day and 48-hr deprivation were summing to increase this effect. A significant increase of ectopic kidneys and internal hydrocephalus resulted from the combination of a 15.8 mg/kg/day dose and 24-hr food deprivation but not from either the drug or food deprivation alone. The test material produced a variety of limb and axial skeletal defects at 15.8 mg/kg/day; the only effect of 14.1 mg/kg/day was delayed ossification of the hallux (Table 2). food deprivation for 48 hr, alone or in combination with the test material, increased the incidence of delayed ossification of small bones but did not alter the frequency of occurrence of the more pronounced skeletal defects, such as reduced or missing limbs or fused vertebrae, in the foetuses of treated animals.

Phenobarbital pretreatment decreased test material-induced resorptions and reductions in foetal body weight and SKF-525A pretreatment significantly increased test material-induced maternal and embryo mortality (Table 3). SKF-525A significantly enhanced the incidence of test material-induced 915.8 mg/kg) ectrodactyly and reduced the percentage of normal foetuses (Table 3). the effect of phenobarbital was not consistent. Administered prior to 17.7 mg/kg test material on day 11, phenobarbitol decreased the percentage of normal mice in soft tissue examination but increased this percentage when given prior to a 18.8 mg/kg dose on day 12 (Table 3). Phenobarbital decreased the incidence of test material-induced (17.7 mg/kg) missing vertebrae and increased the incidence of supernumary ribs.

Table 1: Resorption rate, body weight, and percent normal foetuses in external and soft tissue examinations among offspring of mice treated with dinoseb (gestational days 10,11, & 12) after food deprivation (gestational days 9 or 9 &10)

Dose (mg/kg/day)	Deprivation (hr)	No. of pregnant mice treated [a]	Resorbed or dead (%) [b]	Foetal body weight (g) [b]	External exam (% normal) [b]	Soft tissue exam (% normal) [b]
0	0	14	6.3 (2.2)	1.39 (0.03)	98.7 (0.9)	88.4 (3.9)

0	24	14	11.8 (5.7)	1.36 (0.02)	95.5 (2.0)	77.7 (7.2)
0	48	7	9.1 (3.0)	1.33 (0.02)	96.8 (2.0)	53.5 (8.6) †c
14.1	0	7	4.4 (2.2)	1.27 (0.03)	95.8 (2.2)	83.2 (3.0)
14.1	24	6	6.7 (2.7)	1.24 (0.05)*d	81.6 (14.9)	62.1 (15.0)
14.1	48	7	26.9 (13.7)	1.14 (0.05)*†	78.7 (16.1)	55.1 (14.3)
15.8	0	15	8.9 (2.0)	1.19 (0.03)	73.7 (8.0)*	61.2 (7.4)
15.8	24	14	13.4 (3.5)	1.16 (0.03)*	48.3 (10.0)*†	46.1 (9.4)*
15.8	48	7	20.4 (13.5)	1.16 (0.04)*	71.3 (8.1)*	45.7 (12.9)

[a] All pregnant mice survived
[b] Values are mean response/litter (SE)
[c] Values marked with a dagger (†) differ significantly from those of the non-deprived group that was treated with the same dose of test substance: $p<0.05$
[d] Values marked with an asterisk (*) differ significantly from those of the similarly deprived, no drug group: $p<0.05$

Table 2: Percent incidence of skeletal anomalies among offspring of mice treated with dinoseb (gestational days 10, 11, & 12) after food deprivation (gestational days 9 or 9 & 10)

	Dose (mg/kg/day)								
	0			14.1			15.8		
Type of anomaly	0 (14)	24 (14)	48 (7)	0 (7)	24 (6)	48 (6)	0 (15)	24 (14)	48 (6)
Digital bones (FL)[b] abs or NO [c]	0 [d]	1.2 (1.2)	0	0	5.6 (5.6)	20.0 (16.3)*†c	8.5 (3.1)	18.4 (8.0)*	6.9 (3.1)
Ischium or pubis abs or small	0	1.0 (1.0)	0	2.0 (2.0)	11.1 (11.1)	8.3 (8.3)	27.6 (9.0)*	27.6 (7.1)*	14.0 (5.2)
Femur abs or small	1.4 (1.4)	0	0	2.0 (2.0)	18.0 (13.7)	12.5 (12.5)	19.5 (7.3)*	40.2 (9.8)*†	14.0 (5.2)
Tibia or fibula abs or small	0	0	0	0	18.0 (13.7)	12.5 (12.5)	20.7 (8.2)*	46.2 (10.1)*†	16.4 (5.8)
Digital bones (HL) [f] abs or NO	0	1.2 (1.2)	0	0	12.5 (8.5)	15.8 (12.30	11.4 (5.4)	40.1 (9.4)*†	9.5 (6.0)
Phalanges (HL) abs or NO	18.5 (7.1)	23.3 (7.5)	40.2 (13.0)	37.2 (12.9)	43.2 (18.3)	74.4 (14.0)†	32.3 (6.9)	58.9 (9.9)*†	54.8 (14.4)
Hallux abs or NO	27.0 (8.7)	38.4 (9.3)	70.8 (11.4)†	84.9 (8.9)*	77.9 (11.4)*	97.6 (2.4)	79.8 (5.6)*	80.2 (8.0)*	91.7 (8.3)
Ribs fused	0	8.3 (5.7)	12.2 (12.2)	8.2 (5.3)	5.2 (3.3)	14.0 (8.0)	17.6 (5.6)*	35.7 (7.4)*†	14.3 (9.0)
Vertebrae incomplete	2.4 (2.4)	2.1 (1.4)	16.3 (14.1)	10.8 (4.3)	11.1 (11.1)	29.8 (15.0)	26.3 (6.8)*	37.0 (8.9)*	12.3 (6.8)
Vertebrae fused	0	3.6 (3.6)	6.1 (6.1)	23.0 (12.0)	33.1 (15.0)*	39.4 (14.6)*	59.6 (9.3)*	63.9 (9.9)*	61.4 (10.5)*
Vertebrae split	0	5.7 (3.7)	0	0	2.4 (2.4)	0	17.0 (4.0)*	14.3 (4.7)	11.0 (7.1)
Sternabrae abs or NO	0	2.0 (1.4)	0	0	25.4 (14.0)*†	10.7 (8.2)	18.0 (4.1)*	42.0 (4.8)*†	13.1 (7.9)
Sternabrae split	6.0 (2.9)	20.5 (6.9)	18.2 (8.0)	21.3 (9.2)	2.4 (2.4)	30.2 (8.2)	30.6 (5.6)*	23.7 (4.8)	39.6 (7.8)

[a] The number in parentheses given with the deprivation time is the number of litters examined.
[b] FL = front limb
[c] abs or NO = absent or not ossified
[d] Values are percent incidence mean response/litter (SE)
[e] Values marked with an asterisk (*) differ significantly from those of the similarly deprived, no drug group; $p<0.05$; values marked with a dagger (†) differ significantly from those of the non-deprived group that was treated with the same dose of test material; $p<0.05$
[f] HL = hind limb

Table 3: Resorption rate, body weight, and percent normal foetuses in external and soft tissue examinations among offspring of pregnant mice treated with Phenobarbital or SKF-525A prior to dinoseb treatment.

Pretreatment	Dinoseb (mg/kg/day)	Day of dinoseb treatment	No. of mothers		Resorptions (%) [a]	Foetal body weight (g) [a]	External exam (% normal) [a]	Soft tissue exam (% normal) [a]
			Treated	Surviving				
None	0	-	7	7	5.7 (2.2)	1.38 (0.05)	100.0	100.0
None	17.7	11	7	7	10.3 (4.9)	1.22 (0.02)	79.4 (11.7)	79.5 (5.3)*c
Phenobarbital [b]	17.7	11	7	7	5.6 (2.8)	1.27 (0.02)	96.8 (1.6)	44.2 (4.4)*†c
None	18.8	12	6	6	55.0 (19.0)*	0.79 (0.25)*	98.9 (1.9)	22.2 (14.7)*
Phenobarbital [b]	18.8	12	8	8	8.0 (2.4)†	1.34 (0.03)†	100.0	76.7 (4.4)*†
SKF-525A [d]	15.8	12	7	4	36.0 (12.0)*	1.19 (0.07)	85.4 (12.0)	29.2 (10.5)*
SKF-525A [d]	17.7	12	8	2	NA	NA	NA	NA

[a] Values are mean response/litter (SE)
[b] Administered 50 mg/kg twice daily for 3 days prior to dinoseb treatment

[c] Values marked with an asterisk (*) differ significantly from those of no pretreatment, no dinoseb group: $p<0.05$; values marked with a dagger (†) differ significantly from those of the no pretreatment group that received an equivalent dose of dinoseb: $p<0.05$
[d] Administered 32 mg/kg 1 hr prior to dinoseb treatment.
[e] NA = not analysed because of small number of litters surviving.

Reliability : (2) valid with restrictions
29.01.2003 (28)

Species	:	rabbit
Sex	:	female
Strain	:	New Zealand white
Route of admin.	:	dermal
Exposure period	:	6 hours
Frequency of treatm.	:	daily
Duration of test	:	13 days (days 7 through 19 of pregnancy)
Doses	:	0, 1, 3, 9, 18 mg/kg/day
Control group	:	yes, concurrent no treatment
Method	:	other: EPA, Guidelines for the health assessment of suspect developmental toxicants, Federal Register, 51(185), 24028-34040, 1986
Year	:	1988
GLP	:	no data
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Source: No data Purity: No data

Method : Age at study initiation: Approximately 6 months
Number of animals/dose: 16
Vehicle: None
Clinical observations performed: Bodyweight, rectal temperature, physical signs of adverse effects.
Mating procedures: Females were artificially inseminated with spermatozoa from untreated proven male breeders from the same strain. Each doe was inseminated with a calculated 6.0xE+6 spermatozoa in 0.25 ml saline - diluted semen, which was administered to the females 3 hours after they had been intravenously injected with 20 USP units per kilogram of human chorionic gonadotropin.

Result : RESULTS

NOEL maternal toxicity: 1 mg/kg/day
NOEL developmental toxicity: 1 mg/kg/day

Maternal data:

In this study there were initially 5 groups of animals. The 18 mg/kg/day treatment level proved overly toxic and, since animals werE dying also in the 9 mg/kg/day group, those which had begun or were assigned to the 18 mg/kg/day regimen were reassigned and had their dosage reduced to 9 mg/kg/day. Animals that received 18 mg/k/day did not contribute to the evaluation of the effects of the test substance and were not discussed further.

Mortality: 71% of the animals receiving 9mg/kg/day died during pregnancy. The incidence of maternal deaths was slightly elevated also in the 3 mg/kg/day animals. These deaths were considered compound related because 2 of the animals were observed to have altered respiratory patterns (Table 1).

Number pregnant per dose level: See Table 1

Number aborting: See Table 1

Number of implantations: Not affected by treatment (Table 1).

Body weight: There was a large cumulative average maternal weight loss during the treatment period. from the outset, the high-dose females lost weight at a much greater rate than did those of the other two groups, including the controls, but, after the period of days 13 to 16, there was no further cumulative weight loss in this group.

Food/water consumption: Before treatment with the test substance began, all groups of pregnant rabbits ate approximately the same amount of feed, but, concomitant with treatment, there was a generally dose-related decrement of feed intake for the first week on treatment. there was a severe reduction in the high dose group, and they alone had a rebound of intake that became evidentduring days 13 to 16. Intake was at, or just above, control levels on days 16-20 and after treatment, i.e. days 20-24.

Description, severity, time of onset and duration of clinical signs:

The control and low dose animals had body temperatures that were essentially similar throughout the study. The mid dose animals had temperatures somewhat higher during the first two days and last week of treatment. This phenomenon was accentuated in the high dose animals, which consistently maintained an elevated body temperature until after treatment had ceased, at which time it declined, to come within the range of the other three groups. High dose maternal body temperature reached its maximum on day 8 when it was 103.5°C, trended lower for the next four days, but was again variably elevated to the 103°C range on days 13 and 14.

Fetal data:

Litter size and weights: Fetal body weight was not affected by the treatment and, though those of the high doe group tended to be heavier than the other groups, this is considered to be an effect of the reduced litter size associated with an increased incidence of dead or resorbed fetuses per litter in the high dose group (Table 1).

Number viable: The number of live fetuses was reduced in the high dose group (Table 1)

Grossly visible abnormalities, external, soft tissue and skeletal abnormalities: In the fetuses of high dose mothers, there were significant litter and fetal incidences of cleft palate, microcephaly, hydrocephaly, microphthalmia, and anophthalmia. At least 2 of these effects, i.e. hydrocephaly and anophthalmia, also were present in the fetuses from mothers receiving 3 mg/kg/day (Table 1). At the level of 1 mg/kg/day, none of these effects were evident, although instances did occur in the controls. Fetal effects frequently found in rabbit fetuses from compromised mothers, i.e. abnormal ossification of the axial skeleton, also were evident in the high dose group, but are not clearly dose related in the two lower treatment groups when compared with offspring of the untreated pregnancies.

Test substance : 2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Source: No data
Purity: No data

Table 1: Effects of test substance on maternal status and *in utero* development

	0 mg/kg	1 mg/kg	3 mg/kg	9 mg/kg
# Pregnant rabbits	16	16	16	17
#(%) deaths or moribund sacrifice of pregnant rabbits	2 (12.5)	2 (12.5)	3 (18.75)	12 (70.6)
#(%) aborted	2 (12.5)	4 (25.0)	2 (12.5)	2 (11.8)
#(%) delivered naturally	3 (18.8)	0	0	0
Mean No. implantations ±SD	7.4±3.1	7.3±3.1	9.1±2.5	7.3±1.2
Mean No. live fetuses ±SD	6.5±2.8	7.1±3.1	8.3±2.3	3.3±2.1

No. litters w/ live fetuses	10	10	11	3
Mean male fetal weight (g) ±SD	42.88±9.30	37.65±7.69	37.93±4.46	49.18±5.92
Mean female fetal weight (g) ±SD	41.49±7.66	41.03±7.08	36.12±5.28	48.53±1.40
Mean % live male fetuses/litter ±SD	56.0±16.5	51.3±17.9	42.6±23.3	71.7±30.1
Mean % dead or resorbed/litter ±SD	11.2±13.4	2.5±5.4	8.2±9.6	51.4±35.4
% cleft palate L/F	0/0	0/0	0/0	33/10
% microcephaly L/F	0/0	0/0	0/0	33/10
% hydrocephaly L/F	0/0	0/0	18/2	100/70
% microphthalmia L/F	10/2	0/0	0/0	67/50
% anophthalmia	0/0	0/0	9/1	100/30
% abnormal caudal vertebrae L/F	0/0	10/3	10/1	33/10
% asymmetric thoracic vertebrae L/F	10/2	10/1	18/2	67/20

Conclusion : It is clear that the test substance has the ability to adversely affect emryonic development or even cause fetal death in a well-designed experiment at dosage levels that also adversely affect maternal homeostasis. At the lower treatment level of 1 mg/kg/day, some perturbation of maternal homeostasis is evident, however in utero development is not altered.

This means the test substance does not adversely affect the conceptus in any uniquely targeted manner, but, instead, produces its effects at treatment levels that are also maternally toxic. this is not to be construed as saying that maternal toxicity is causing the adverse effects, and, in fact, the assumed mechanism of action of the test substance (uncoupling of oxidated phosphorylation) is well known to adversely affect embryonic development.

Reliability : (2) valid with restrictions
17.02.2003 (23)

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period : Day 6 through 15 of gestation
Frequency of treatm. : daily
Duration of test :
Doses :
Control group : yes
Method : other:
Year : 1982
GLP : no data
Test substance : 2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Purity: 95.0%
Stock No.: ACR 133942
Source: Dow Chemical Company

Method : a) Pseudopregnancy: Induced by stimulating the uterine cervical region of the rat with the introduction of a vibrating fiber glass in to the vagina during the proestrus and astrus stages of the reproductive cycle. Day 1 of pseudopregnancy was designated as the first day when leukocytes appeared predominantly in the vaginal smear. Decidual cell reaction of the uteri was induced by surgical traumatization of both horns at Day 4 of pseudopregnancy. Feeding of test material to the decidualized pseudopregnant rats was conducted from the period from Days 6-10 of pseudopregnancy. Animals were sacrificed on Day 10 of pseudopregnancy.

Doses: 0, 25, 50, 100, 200, 250, 350, 500, 750 ppm.

Parameters assessed during study: Body weight, uterine horn weight, uterine protein and glycogen levels determined.

b) Pregnancy: Female rat in estrus stage housed with a fertile male rat. The day on which sperm were first observed in the vaginal lavage was considered as Day 1 of gestation. Feeding of the test material was conducted from Days 6-15 of pregnancy.

Parameters assessed during study: Number of implantation sites in the pregnant rat counted at Day 6 of pregnancy. On Day 12 of pregnancy, laparotomy was performed on the same rats on which the implantation sites were previously counted on Day 6 and once again the number of implantation sites were counted. The percentage of embryo survival rate at day 12 was calculated. On the day (Days 20-23) of parturition in pregnant animals carried to term and whose implantation sites were previosly determined, the number of live and dead fetuses was recorded and the dams were weighed. The live fetuses were examined for external anomalies. The fetuses were also weighed.

In a further experiment, groups of pregnant rats were sacrificed at Day 16 of pregnancy. The placentae from live fetuses were removed for protein and glycogen analyses.

Statistical methods: The degree of significance between the means of the treatment groups at increasing dosages was accounted for by one-way analysis of varience (ANOVA). The statistical significance between experimental and control groups was determined by Student's t-test.

Remarks:

Test animals: Only those rats (weighing between 200 and 300 g) which exhibited two consecutive 4- to 5-day estrous cycles were randomly selected for the experiments. 10 rats used per dose.

Result : Decidualized pseudopregnant (DCR) studies:

Actual doses (mg/kg b.w./day): 0, 1.6 (25 ppm), 3.6 (50 ppm), 4.9 (100 ppm), 8.9 (200 ppm), 12.8 (350 ppm), 13.8 (500 ppm) and 24.3 (750 ppm)

Bodyweight: 750 ppm group significantly lower ($p<0.05$) from the control.

Clinical signs: Lethargy and ataxia were prominent in DCR animals exposed to 750 ppm.

Necropsy: Animals fed 500 and 750 ppm revealed jaundiced uteri. Mesenteric fatty tissues of the uterus were tinged with a light yellowish coloration, probably due to deposition of the test material which is a powerful chromophore. There was no significant reduction in absolute uterine wet weight and relative uterine wet weight at all doses up to and including 500 ppm. Both the absolute and relative uterine wet weights were significantly lower ($p<0.05$) in the 750 ppm dose group. A decrease in DCR uterine response was also noted at 750 ppm. Uterine protein and glycogen levels were reduced significantly ($p<0.05$) when compared with dosage levels.

Pregnancy studies:

Actual doses (mg/kg b.w./day): 0, 3.26 (50 ppm), 6.9 (100 ppm), 9.23 (150 ppm), 10.86 (200 ppm), 9.38 (250 ppm), 9.49 (300 ppm) and 8.6 (350 ppm)

Bodyweight: Reductions in the body weight gain were prominent in the 150 ppm test group. These reductions were more pronounced in the high-dosed groups of 200, 250, 300 and 350 ppm.

Clinical signs: Toxic symptoms such as ataxia and lethargy were displayed

by all the pregnant rats fed 200, 250, 300 and 350 ppm of test material.

Necropsy: Placental protein and placental glycogen concentrations were decreased in 200, 250 and 300 ppm dose groups. Since at higher doses prominent placental resorption was induced, the placental biochemical parameters were not examined at these dosages. As with the DCR rats, there was an accumulation of yellowish fluid in the surrounding fatty tissues of the ovary and the uterus.

Fetal effects: Following exposure to the test material from Days 6-11 of pregnancy, the number of conceptuses per litter on Day 12 was significantly different from control values ($p<0.05$). There were dramatic embryotoxic effects as manifested by resorption of implantation sites. In comparison with the control, decreases in fetal survival were seen in exposed rats. Fetal birth weight was markedly reduced by treatment ($p<0.05$). A major malformation was observed in the 200 ppm experimental group. Hypoplastic tail formation was observed in 8 of 62 fetuses examined at this concentration. The incidence of this anomaly was observed was 33.3% or 2 of 6 litters. No incidence of this anomaly was observed in the concurrent control or in litters from rats exposed to the test material at 150 ppm or below.

Table 1: Effect of dinoseb on maternal weight

Dietary exposure [a] (ppm)	Body weight at Day 6 [b] (g)	Body weight gain during gestation (g)	
		Days 6-12 [b]	Days 6-15 [b]
0	228±5	+23±4	+38±4
50	256±5	+22±2	+38±3
100	240±8	+19±1	+40±5
150	220±3	-4±3*	+3±3*
200	252±7	-14±3*	-30±6*
250	298±10	-20±5*	-52±5*
300	293±7	-51±7*	-74±9*
350	236±3	-37±7*	-58±5*

[a] Dinoseb administered from Days 6 through 15 of gestation
[b] Mean ±SE, for results of 6 rats
* Significantly different from the control value using Student's t test ($p<0.05$)

Table 2: Effect of dinoseb on pregnancy performance

Dietary treatment [a] (ppm)	Daily intake of dinoseb (mg/kg/day)	No. in litter	Implantations at Day 6 per dam	No. of conceptuses at Day 12	% of embryo survival rate per litter at Day 12 [b]	% of fetal survival rate per litter at birth [c]	Fetal birth weight per litter (g)
0	-	6	12.5±1.4[d]	12.5±1.4	100±0.0	80.12±7.59	7.20±0.30
50	3.26±0.09	6	11.7±1.9	11.7±1.9	100±0.0	83.31±12.56	7.13±0.27
100	6.90±0.20	6	13.7±1.1	13.7±1.1	100±0.0	63.09±6.05	6.78±0.14
150	9.23±1.02	6	14.3±0.7	14.3±0.7	100±0.0	45.94±11.56*	6.85±0.41
200	10.86±1.33	6	13.2±1.4	9.7±1.1	75±9.0*	53.06±9.20*	6.43±0.18*
250	9.38±2.05	6	12.3±0.7	7.3±2.6	56±22.3*	16.34±12.32*	-
300	9.49±1.46	6	14.8±2.1	4.9±3.5	33±21.0*	10.81±5.56*	-
350	8.60±1.57	6	12.2±0.4	0.0±0.0	0±0.0	0±0.0	-
ANOVA				p<0.05	p<0.05	p<0.05	p<0.05

[a] Dinoseb administered from Days 6 through 15 of pregnancy
[b] The ratio of the number of surviving embryos per ltter at day 12 to that at Day 6 as expressed in percentage
[c] The ratio of the number of live fetuses at birth to the number of implantation sites counted at Day 6 as expressed in percentage
[d] All results expressed as Mean±SE
* Significantly different from control using Student's t test ($p<0.05$)

Reliability : (2) valid with restrictions
17.02.2003

(31)

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	i.p.
Exposure period	:	days 10-12 of gestation
Frequency of treatm.	:	daily
Duration of test	:	
Doses	:	0, 6.3, 8.0, 9.0, 11.2, 12.5, 15.8 mg/kg/day
Control group	:	yes, concurrent vehicle
Method	:	
Year	:	1980
GLP	:	no
Test substance	:	2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7) Source: Dow Chemical Company Lot No.: 7200206

Method : Type: One generation

Statistical methods: Data were analysed statistically by analysis of variance, completely random design. Treatment differences were detected by the least significant difference test, and $p<0.05$ was used as the criterion of significance: Steel, R.G.D. & Torrie, H.H., Principles and Procedures of Statistics, McGraw-Hill Book Company, Inc., New York, 1960.

Test animals: Timed-pregnant rats were obtained on day 2 of gestation.

Standardization of litters: Litters were normalized to 5 males and 5 females on day 1 postpartum.

Experiments were conducted with offspring at 21 day of gestation or at 1, 7 or 42 days postpartum.

Fetuses used at day 21 of gestation were removed by cesarean section. Numbers of live, dead and resorbed fetuses were recorded. Fetuses were examined under a dissecting microscope for external anomalies. Offspring were also examined under a dissecting microscope for visceral anomalies at gestation day 21 as well as at 1, 7 and 42 days postpartum.

Tissues from offspring at 21 days of gestation (liver, kidney, ureter, heart, lung) and at 1 and 42 days postpartum (liver, kidney, ureter) were used for histological examinations.

Renal function of rats treated prenatally with dinoseb (0 or 9.0 mg/kg/day) was determined both in vitro and in vivo.

Result : Pregnant rats given ip injections of dinoseb at 11.2, 12.5 or 15.8 mg/kg/day all died within 1 week of treatment termination (Table 1). The mortality rate for pregnant rats treated at 9.0 mg/kg/day was approximately 20%. Dinoseb did not cuase maternal death when administered at 8.0 mg/kg/day or less.

Treatment with dinoseb did not affect the number of live fetuses or the resorption rate in surviving dams (Table 2). Fetal length was reduced by dinoseb at 9.0 mg/kg/day and fetal weight was decreased by 8.0 or 9.0 mg/kg/day (Table 2). Rats treated prenatally with 9.0 mg/kg/day weighed less than controls at 1 and 7 days postpartum (Table 3). However body weights of control and dinoseb treated rats were not significanlty different at 42 days of age (Table 3).

Prenatal exposure to dinoseb did not affect the liver weight/body weight ratio (Table 3) or the gross appearance of liver. However, hepatic histopathologic alterations were produced by dinoseb. Livers from fetuses

in the 8.0 or 9.0 mg/kg/day treatment group had many cells that were vacuolated and necrotic at 42 days postpartum. Hepatocytes were disrupted; the nucleus was absent from some cells and pyknotic or karyorrhetic in others. Cellular swelling caused sinusoids to become less apparent. Lesions were most extensive at the periphary of the lobules.

Prenatal treatment with dinoseb did not affect the organ weight/body weight ratios of fetal heart or lung.

The kidney weight/body weight ratio was not affected by dinoseb (Table 3). However, approximately 40% of near-term fetuses treated with dinoseb (8.0 or 9.0 mg/kg/day) had dilated renal pelves and/or ureters when examined grossly. Histological examination of kidneys from near-term and 1 day old rats treated prenatally with dinoseb revealed dilation of the renal pelvis and tubules (particularly distal tubules and collecting ducts). At these ages, mesenchymal tissue was more prevalent in kidneys from dinoseb treated rats. The transitional epithelium of ureters from near-term and 1 day old rats treated with dinoseb was vacuolated. The incidence and severty of lesions in kidneys and ureters from dinoseb treated rats decreased with age. At 42 days of age, gross examination of kidneys and ureters did not reveal any dilated ureters and only 3 of 28 animals treated prenatally with dinoseb (9.0 mg/kg/day) had dilated renal pelves (none in controls). Microscopic differences between ureters and kidneys (with nondilated pelves) from 42 day old dinoseb treated and control rats were not detected.

Although renal histopathologic changes were observed in near term and neonatal rats, prenatal treatment with dinoseb did not affect any renal functions tested.

Table 1: Mortality of pregnant rats after treatment with dinoseb [a]

Treatment (mg/kg/day)	No. of rats treated	No. of rats surviving
0	12	12
6.3	6	6
8.0	10	10
9.0	16	13
11.2	6	0
12.5	6	0
15.8	4	0

[a] Rats received ip injections of dinoseb on days 10-12.

Table 2: Resorption rate and size of fetuses from pregnant rats treated with dinoseb [a]

Treatment (mg/kg/day)	No. of live fetuses	Resorbed or dead fetuses (%)	Fetal body weight (g)	Fetal crown-rump length (cm)
0	13±1	5.8±2.3	5.82±0.08	3.9±0.1
6.3	11±1	8.6±3.8	5.91±0.10	3.9±0.1
8.0	12±1	4.9±1.5	5.44±0.06 [b]	3.9±0.1
9.0	11±1	7.1±2.2	4.96±0.07 [b]	3.4±0.2 [b]

[a] Rats received ip injections of dinoseb on days 10-12. Rats were killed at day 21 of gestation. Values are mean per litter ± SEM for at least 6 litters.
[b] Significantly different from control value, $p<0.05$

Table 3: Body weight, Liver weight/body weight ratio and kidney weight/body weight ratio for rats treated prenatally with dinoseb [a]

Treatment (mg/kg/day)	Age (days)	Body weight (g)	Liver weight/body weight (x 100)	Kidney weight/body weight (x 100)
0	1	6.7±0.1	5.48±0.15	0.91±0.02
9.0	1	5.9±0.2 [b]	5.63±0.21	0.93±0.02

0	7	15.6±0.4	2.97±0.04	1.10±0.02
9.0	7	13.8±0.6 [b]	3.19±0.09	1.16±0.03
0	42 [c]	143.5±9.1	4.58±0.23	0.93±0.03
9.0	42 [c]	139.4±7.7	5.04±0.18	0.95±0.05

[a] Pregnant rats received ip injections of dinoseb on gestational days 10-12. Values are means± SEM for at least 8 animals.
[b] Significantly different from control value, $p<0.05$
[c] Values are for females rats. Body weights were 171.8±12.4 and 174.9±18.6 g for control and dinoseb treated male rats, respectively.

Reliability : (2) valid with restrictions
04.03.2003 (26)

Species : rat
Sex : female
Strain : other: CD
Route of admin. :
Exposure period :
Frequency of treatm. :
Duration of test :
Doses :
Control group :
Method :
Year : 1986
GLP : no data
Test substance : 2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Source: Serva, Heidelberg
Purity: Analytical grade

Method : Animals: Virgin CD rats, 170±10 g

Mating: Caged overnight with males of proven fertility. Confirmed by presence of spermatozoa in the vaginal smear the following morning (day 1 of pregnancy).

Experiment 1:

Route of administration: Oral gavage

Doses: 0, 2.5, 5, 10, 15 mg/kg

Exposure period: Days 6-15 of gestation

Frequency: Daily

The test material was freshly dissolved in corn oil in such concentration as to administer 0.5 ml/100 g of bodyweight.

Experiment 2:

Route of administration: Feed

Doses: 200 ppm (chosen to give a daily intake of approx 15 mg/kg)

Exposure period: days 6-15 of gestation

Frequency: Daily

Because in a preliminary test a diminuation was observed in the food intake of females on a diet containing dinoseb, a control group supplied with a quantitiy of food about equal to the average daily consumption of the

experimental group females was set up. Control animals received a regular diet ad libitum.

Experiment 3:

(a)

Route of administration: Gavage

Doses: 7.5, 10 mg/kg

Exposure period: Days 6-13 of gestation

Frequency: Twice daily (9 a.m and 6 p.m.) to give a total daily dosage of 15 and 20 mg/kg.

(b)

Route of administration: Gavage

Doses: 15 mg/kg

Exposure period: Days 6-13 of gestation

Frequency: Daily

The dinoseb was dissolved in 1N NaOH and titrated to pH 7 with HCl.

(c)

Repeat of Experiment 2

Parameters recorded: Bodyweight of pregnant females measured every 3 days. All females were killed on the 21st day of gestation. After rough necroscopic examination, the following parameters were recorded: number of corpora lutea, number of implantations, number of resorptions and live fetuses, weight of live fetuses and eventual external malformations. Skeletal development was also evaluated.

Statistical evaluation: Maternal body weights, food consuption, corpora lutea, implantations, litter size and fetal weight were analyzed by one way analysis of variance followed by Dunnet's t-test. Incidence data were evaluated by the chi square test. The level of significance chosen for all experimental parameters was p=0.05.

Result : Experiment 1:

During the treatment period, maternal body weight gain was reduced among animals receiving 10 and 15 mg/kg (Table 1). At 15 mg/kg, 4/15 females died within the first five days of treatment. No differences were noticed with respect to the control as far as the number of implantations, resorptions and live fetuses were concerned. The mean fetal weight was significantly reduced only at the highest dose levels. One fetuswith cerebral haemorrhage was found in the group treated with 5 mg/kg and two fetuses with visceral malformations among the controls. Skeletal examination revealed only one plurimalformated fetus at the highest dose level. A dose related increase of the skeletal anomalies (in particular of the extra ribs) was observed, with values significantly superior to the control starting from teh 10 mg/kg dose. A significant delay in ossification was present in fetuses belonging to the group treated with the highest dose, at metacarpus and sternebrae level.

Experiment 2:

Dinoseb adminsitration in the diet caused a noticeable reduction of weight gain during the treatment period, followed by a significant increase in the post treatment phase of pregnancy. A similar trend, if less noticeable, was found in animals fed with a reduced diet. No animal deaths were oserved (Table 3). The average daily intake of food and dinoseb is reported in Table 4. Compared to the control, dinoseb exposure did not cause any variation in the number of implantations, live fetuses or resorptions; whereas in animals with a reduced diet, an increase in the rate of resorptions was found. The fetuses from the dinoseb exposed dams weighed significantly less than fetuses from the control animals and females maintained on a reduced diet (Table 3). No difference in the frequency of major malformations, anomalies or delays in ossification between the control and the group fed with reduced diet was observed, whereas 14 fetuses form 6 litters exposed to 200 ppm of dinoseb showed a severe form of microphthalmia, unilateral in 13 cases and bilateral in one case. Furthermore, the frequency of anomalies was significantly higher in this group.

Experiment 3:

The administration of repeated doses of dinoseb caused the death of 3 dams at 7.5 mg/kg and 4 dams at 10 mg/kg. 3 other dams died during treatment with dinoseb (15mg/kg) dissolved in NaOH (Table 6). In all of the groups exposed to dinoseb, the maternal weight change was adversely affected during the treatment period, especially in the group exposed via the diet. In this latter group, during post treatment period, the maternal weight gain was greater than in the control and in the other groups. In all treated groups, food consumption was reduced in comparison with the control during the treatment period (Table 4).

Dinoseb treatment does not influence implantation, nor does it cause an increase in embryolethality. A reduction of average fetal weight was observed in all the groups, particularly with treatment through the diet (Table 6).

A fetus was found with microphthalmia in the group treated with 15 mg/kg in NaOH and a fetus with hydronephrosis (severe pelvis dilatation with marked reduction of renal cortex) in the group treated twice a day with 7.5 mg/kg. At 200 ppm in the diet 7 (9.3%) fetuses in 3 litters were affected by microphthalmia (5 unilateral and 2 bilateral cases). Further more, a significant increase of skeletal anomalies was noticed in all of the treated groups (Table 7). a significant delay in some ossification centra was observed in fetuses from dams exposed to dinoseb in diet or gavaged with 15 mg/kg in NaOH.

Table 1: Reproductive performance of rat females treated by gavage with dinoseb dissolved in corn oil

	Doses (mg/kg/day)				
	0	2.5	5	10	15
Females					
Mated	15	17	16	15	15
Dead	-	-	-	-	4
Pregnant at term	13	15	15	15	11
Maternal weight gain (g)					
Days 6-15 (mean ± SD)	41.2±9.6	34.6±7.6	35.4±5.4	28.9±10.8 [b]	20.3±12.5 [b]
Days 15-21 (mean ± SD)	65.0±12.6	63.7±14.7	62.3±8.4	58.8±12.4	66.4±11.4
No. of corpora lutea (mean ± SD)	13.7±1.8	13.8±1.2	13.8±1.7	13.1±1.1	14.8±1.7
No. implantations (mean ± SD)	13.5±2.0	12.4±2.9	13.2±1.3	12.2±1.4	13.5±1.1
No. live fetuses (mean ± SD)	13.0±1.6	11.9±2.8	12.3±1.9	11.3±1.9	13.2±1.1
No. of resorptions	7	8	14	13	3
% post-implantation loss (mean ± SD)	3.6±5.3	4.0±5.5	7.2±9.6	7.5±8.4	1.9±3.2
Dams with resorptions (%)	5 (38.4)	6 (40.0)	8 (53.3)	9 (60.0)	3 (27.7)

Fetal weight (g, mean ±SD)	4.1±0.3	4.0±0.1	4.0±0.2	3.9±0.2	3.7±0.1 [a]

[a] $p<0.05$
[b] $p<0.01$

Table 2: Teratological defects in rat fetuses following maternal treatment with dinoseb by gavage

	Doses (mg/kg/day)				
	0	2.5	5	10	15
Visceral malformations					
Affected fetuses	2/86	0/89	1/93	0/84	0/73
Affected litters	2/13	0/15	1/15	0/15	0/11
Cerebral hemorrhage	-	-	1	-	-
Diaphragmatic hernia	1	-	-	-	-
Ectopic adrenals	1	-	-	-	-
Ectopic ovary	1	-	-	-	-
Hydronephrosis	1	-	-	-	-
Skeletal malformations					
Affected fetuses	0/78 [c]	0/90	0/92	0/81 [d]	1/72 [e]
Affected litters	0/12	0/15	0/15	0/14	1/11
Agenesis 1st & 4th thoracic vertebral body	-	-	-	-	1
Fused ribs	-	-	-	-	1
Scoliosis	-	-	-	-	1
Sternebrae fused	-	-	-	-	1
Sternum bifurcated	-	-	-	-	1
Visceral anomalies					
Affected fetuses	1/86	1/89	1/93	0/84	0/73
Affected litters	1/13	1/15	1/15	0/15	0/11
Renal pelvis dilatation	1	1	1	-	-
Skeletal anomalies					
Affected fetuses	24/78 [c]	20/90	32/92	35/81 [d]	37/72 [a, e]
Affected litters	10/12	10/15	13/15	13/14	11/11
Asymmetrical sternebrae	4	6	12	6	5
Emisternebrae	7	2	4	3	2
Extra ribs	12	7	17	25 [a]	27
Reduced pelvic girdle ossification	-	-	-	1	1
Reduced skull ossification	3	1	5	4	4
Reduced vertebral body ossification	-	-	-	1	-
Sternebrae bipartite	2	3	1	-	-
Vertebrae bipartite	-	2	3	-	2
Wavy ribs	1	-	2	6	2
No. of ossified (mean ± SD)					
Caudal vertebrae	3.8±0.4	3.6±0.5	3.5±0.4	3.6±0.5	3.8±0.3
Metacarpals	3.9±0.1	3.8±0.3	3.8±0.1	3.8±0.2	3.6±0.2 [b]
Sternebrae	5.7±0.2	5.8±0.2	5.7±0.3	5.8±0.3	5.4±0.3 [a]

[a] $p<0.05$
[b] $p<0.01$
[c] 5, [d] 5 and [e] 1 fetus lost during preparation.

Table 3: Reproductive performances of rat females exposed to dinoseb in the diet

	Control	Restricted diet	200 ppm
Females			
Mated	10	10	15
Pregnant at term	8	10	14
Maternal weight gain (g)			
Days 6-15 (mean ± SD)	46.3±9.6	17.2±11.5 [a]	-5.7±9.5 [a, b]
Days 15-21 (mean ± SD)	62.3±5.2	81.1±10.9 [a]	80.2±8.7 [c]
No. of corpora lutea (mean ± SD)	16.1±2.4	15.5±1.9	14.5±1.2
No. implantations (mean ± SD)	14.2±1.1	13.9±2.5	13.0±2.1
No. live fetuses (mean ± SD)	13.5±1.6	11.9±2.6	12.2±2.5
No. of resorptions	6	20	11
% post-implantation loss (mean ± SD)	5.3±7.4	14.5±15.2	6.6±8.7

Dams with resorptions (%)	6 (50.0)	8 (80.0)	6 (42.8)
Fetal weight (g, mean ±SD)	4.0±0.1	3.8±0.3	3.4±0.3 [a, b]

[a] = significantly different from control ($p<0.01$)
[b] = significantly different from restricted diet ($p<0.01$)
[c] = significantly different from control ($p<0.05$)

Table 4: Daily food and dinoseb intake

	Average food intake during treatment (g ± SD)	Average food intake after treatment (g ± SD)	Average daily dinoseb intake (mg/kg)
Experiment 2			
Control	23.8±1.2	24.3±2.7	-
200 ppm in diet	19.4±3.1 [b]	28.5±4.2 [b]	17.9
Restricted diet	19.4±3.1 [b]	25.8±2.6	
Experiment 3			
Control	22.9±0.6	24.4±1.2	-
7.5 [c] mg/kg	19.3±1.4 [b]	28.1±1.9 [a]	15
10 [c] mg/kg	18.9±1.0 [b]	24.9±2.0	20
15 [d] mg/kg	22.8±2.7	28.2±2.3 [a]	15
200 ppm in diet	22.5±2.9	36.3±3.7 [b]	18.7

[a] = $p<0.05$
[b] = $p<0.01$
[c] = twice daily in corn oil
[d] = dissolved in NaOH

Table 5: Teratological defects in rat fetuses following maternal exposure to dinoseb in diet

	Control	Restricted diet	200 ppm
Visceral malformations			
Affected fetuses	0/52	0/60	15/83 [a]
Affected litters	0/8	0/10	6/14
Bilateral microphthalmia	-	-	1
Unilateral microphthalmia	-	-	13 [a]
Hydronephrosis	-	-	1
Skeletal malformations			
Affected fetuses	0/56	1/59	0/88
Affected litters	0/8	1/10	0/14
Bifurcated ribs	-	1	-
Visceral anomalies			
Affected fetuses	1/52	0/60	1/83
Affected litters	1/8	0/10	1/14
Renal pelvis dilatation	1	-	1
Skeletal anomalies			
Affected fetuses	19/56	11/59	80/88 [a]
Affected litters	7/8	7/10	14/14
Asymmetrical sternebrae	-	2	3
Emisternebrae	3	1	3
Extra ribs	5	4	79 [a]
Reduced ossification 13th rib	1	-	-
Sternebrae bipartite	1	1	1
Vertebrae bipartite	1	-	2
Wavy ribs	-	-	2
No. of ossified (mean ± SD)			
Caudal vertebrae	3.9±0.3	3.6±0.6	3.8±0.4
Metacarpals	3.8±0.1	3.8±0.2	3.6±0.3
Sternabrae	5.6±0.3	5.4±0.5	5.3±0.5

[a] = $p<0.01$ from control and restricted diet

Table 6: Reproductive performances of rat females given dinoseb with different route of administration

	Gavage (doses mg/kg/day)				Diet
	0	7.5 [c]	10 [c]	15 [d]	200 ppm
Females					

Mated	10	13	10	16	11
Dead	-	3	4	3	-
Pregnant at term	10	8	5	13	10
Maternal weight gain (g)					
Days 6-15 (mean ± SD)	34.4±8.6	25.3±3.2 [a]	17.6±7.7 [b]	13.6±9.8 [b]	-8.3±7.9 [b]
Days 15-21 (mean ± SD)	61.6±9.0	69.3±13.2	59.8±11.9	73.7±9.7 [b]	107.1±16.2 [b]
No. of corpora lutea (mean ± SD)	13.5±1.2	13.5±2.0	12.6±1.9	16.2±2.5	16.8±2.4
No. implantations (mean ± SD)	12.3±2.4	13.5±2.0	11.2±3.4	14.6±1.9	15.7±2.5
No. live fetuses (mean ± SD)	11.8±2.3	12.7±1.9	11.0±3.3	13.4±1.9	15.4±2.2
No. of resorptions	5	6	1	15	3
% post-implantation loss (mean ± SD)	3.9±4.2	5.3±7.4	1.7±3.7	7.7±6.5	1.6±3.6
Dams with resorptions (%)	5 (50.0)	3 (37.5)	1 (20.0)	9 (69.2)	2 (20.0)
Fetal weight (g, mean ±SD)	4.0±0.1	3.8±0.1 [a]	3.5±0.2 [b]	3.5±0.2 [b]	3.3±0.1 [b]

[a] = $p<0.05$
[b] = $p<0.01$
[c] = twice daily in corn oil
[d] = dissolved in NaOH

Table 7: Teratological defects in rat fetuses following different route of maternal exposure to dinoseb

	Gavage (doses mg/kg/day)				Diet
	0	7.5 [c]	10 [c]	15 [d]	200 ppm
Visceral malformations					
Affected fetuses	0/59	1/52	0/28	1/85	7/75 [a]
Affected litters	0/10	1/8	0/5	1/13	3/10
Bilateral microphthalmia	-	-	-	1	2
Unilateral microphthalmia	-	-	-	-	5
Hydronephrosis	-	1	-	-	-
Skeletal malformations					
Affected fetuses	0/59	1/50	0/27	0/90	0/79
Affected litters	0/10	1/8	0/5	0/13	0/10
Bifurcated ribs	-	1	-	-	-
Visceral anomalies					
Affected fetuses	0/59	0/52	0/28	1/85	0/75
Affected litters	0/10	0/8	0/5	1/13	0/10
Renal pelvis dilatation	-	-	-	1	-
Skeletal anomalies					
Affected fetuses	14/59	16/50	23/27 [b]	37/90 [b]	42/79 [b]
Affected litters	7/10	5/8	5/5	12/13	10/10
Asymmetrical sternebrae	3	2	6 [a]	7	8
Emisternebrae	1	-	4	6	7
Extra ribs	4	14 [b]	21 [b]	24 [b]	29 [b]
Reduced skull ossification	5	-	-	-	-
Sternebrae bipartite	-	-	2	-	-
Unossified sacral vertebrae	-	-	-	-	1
Vertebrae bipartite	1	-	1	7	1
Wavy ribs	2	-	-	3	3
No. of ossified (mean ± SD)					
Caudal vertebrae	3.7±0.5	3.5±0.4	3.4±0.5	3.2±0.4 [b]	2.9±0.5 [b]
Metacarpals	3.6±0.2	3.8±0.2	3.5±0.4	3.5±0.3	3.2±0.3 [b]
Sternabrae	5.7±0.3	5.8±0.2	5.3±0.5	5.2±0.7 [a]	5.3±0.5 [a]

[a] = $p<0.05$
[b] = $p<0.01$
[c] = twice daily in corn oil
[d] = dissolved in NaOH

Conclusion : The administration of dinoseb in the diet resulted in a specific teratogenic effect (microphthalmia) at a dose level (200 ppm) which also induced maternal toxicity.

The administration of dinoseb by oral gavage induced maternal toxicity and embryotoxicity (reduced fetal weight and increased frequency of extra ribs) at the highest doses with no teratogenic effects.

Reliability : (2) valid with restrictions
05.03.2003 (17)

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. :
Exposure period :
Frequency of treatm. :
Duration of test :
Doses :
Control group :
Method :
Year : 1988
GLP : no data
Test substance : 2-sec-butyl-4,6-dinitrophenol (CAS No. 88-85-7)
Source: Dow Chemical, Midland, MI
Purity: 97.3%
Lot No. AGR 133942

Method : Animals: Timed pregnant rats received by the laboratory on gestation day 2 (sperm positive day = day 1).

Experiment 1:

Route of administration: ip injection

Doses: 0, 4.5, 6.0, 7.5, 9.0, 12.0, 18.0 mg/kg

Exposure period: Days 10-12 or 11-13 of gestation

Frequency: Daily

Injection volume: 5 ml/kg b.w. (Test material was dissolved in 0.1N NaOH and titrated to pH 7.4 with 0.1N HCl).

Controls: injected daily with saline on gestation days 10-13.

Duration: Pregnant dams were killed on gestation day 21

Parameters recorded: Numbers of live and dead fetuses and resorptions from the gravid uterus were recorded. Live fetuses were removed and weighed as a litter. Fetuses were examined for dilated renal pelvis and other soft tissue anomalies.

Experiment 2:

Route of administration: ip injection

Doses: 0, 8, 10.5 mg/kg

Exposure period: Days 10-12 of gestation

Frequency: Daily

Injection volume: 5 ml/kg b.w.

Controls: injected daily with saline on gestation days 10-13.

Duration: Dams gave birth on gestation day 22 or 23. The following day was designated as postnatal day (PD) 1. Final testing on offspring was conducted on PD30.

Standardization of litters: On PD 1 all litters within a dose group were removed from their dams and segregated by sex. Five male and five female pups were then selected randomly and returned to each dam.

Parameters recorded: Assessments of renal function were made on juvenie rats from just after birth until after weaning. Renal cortical transport, basal urinary parameters and urine concentrating ability were evaluated.

Statistical methods: Differences between control and treated groups and dose-response trends were analyzed using the general linear models procedures available on the Statistical Analysis System (Helwig, J. & Council, K.A. (Eds.), SAS User's Guide, SAS Institute, Cary, NC, 1979). Differences in percentage of fetuses with dilated renal pelvis were analyzed using Fisher's exact test.

Result : Maternal mortality: The lowest dose at which maternal mortality was observed was 8.0 mg/kg.day, 10.5 mg/kg/day was an approximate LD50 and 18 mg/kg/day was lethal to 100% of the dams (Table 1).

Prenatal effects: Embryolethality was not increased by dinoseb treatment, but fetal weight decreased with increasing dosage (Table 2). Mean kidney score, a measure of renal pelvis dilation, was not affected by dinoseb treatment, but the percentage of fetuses with severely dilated renal pelvis (kidney score greater than 5) was significantly increased in fetuses of dams injected with 6.0 and 12.0 mg/kg on gestation days 10-12. Although the frequency of dilated renal pelvis tended to increase with increasing dose, this relationship was not statistically significant. Since elevated frequencies of dilated renal pelvis seemed to occur at doses which were maternally toxic, 10.5 mg/kg/day (approximate maternal LD50) and 8.0 mg/kg/day (approximate highest dose which was not maternally lethal) were chosen as the doses to administer for the postnatal study of renal function.

Postnatal observations: Average litter size and birth weight were not affected by prenatal dinoseb treatment (Table 3), but a significant decrease in body weight was noted on PD30 in rats from the high-dose group, and kidney/body weight ratiowas increased in this group (Table 4). On the other hand, body weight was increased on PD14 in pups from the 8 mg/kg group (Table 5).

Only a few cases of dilated renal pelvis were observed postnatally. Three rats (5%) with mild unilateral dilated renal pelvis were observed in the control group on PD31. In the 8 mg/kg/day group, 6% of pups had unilateral dilated renal pelvis and 6% had bilateral dilated renal pelvis on PD3. By PD31, only on erat (2%) in the 8 mg/kg group had severe unilateral dilated renal pelvis, and two had mild unilateral dilated renal pelvis. One rat (5%) in the 10 mg/kg group had unilateraldilated renal pelvis on PD31. Presence of dilated renal pelvis was not correlated with any alterations in renal function, with the exception of one of the pups in the 8 mg/kg group which had a decreased ability to accumulate AIB on PD3.

Proximal tubule function was assessed by measuring the accumulation of PAH, TEA, AIP and MGP into renal cortical slices. There was a significant increase in the accumulation of PAH into kidney slices from the 10.5 mg/kg group on PD3 and PD31, but there was a significant decrease in PAH accumulation in the 8.0 mg/kg group on PD31. There were no changes in the accumulation of other substrates (Table 6).

Urine concentrating ability was measured by DDAVP challenge (sucklings) or water deprivation (weanlings). By PD14, rats in the 10.5 mg/kg group were inable to concentrate urine as well as controls (Table 5). There were no differences between PD30 control and treated rats in response to 24 hr of water deprivation, except that rats of the 8.0 mg/kg group expressed significantly less urine than controls (Table 4).

Few other urinary parameters were affected in pups prenatally exposed to dinoseb.

Table 1: Maternal mortality in rats following dinoseb injection

Dose (mg/kg/day) [a]	Number injected	Number surviving	% Dead
0	46	46	0
4.5	11	11	0
6.0	11	11	0
7.5	11	11	0
8.0	27	26	4
9.0	11	9	18
10.5	19	10	47
12.0	13	4	69
18.0	12	0	100

[a] dinoseb was injected on gestation days10-12 or 11-13

Table 2: Effects of maternal dinoseb administration on term rat fetuses

Dosage (mg/kg/day)	No. litters	Avg. No. implants	Avg. no. live fetuses	% Dead & resorbed fetuses	Avg. fetal wt (g)	Kidney score [a]	% Fetuses with kidney score ≥5
0	13	12.8±0.8	12.2±0.7	5.2±1.6	3.59±0.04	2.9±0.1	5.7
Dosed d10-12							
4.5	4	8.5±3.2	7.8±2.8	5.4±3.4	3.43±0.22	2.9±0.4	1.9
6.0	4	9.8±2.8	8.5±2.4	12.0±5.2	3.53±0.15	3.7±0.7	32.4 [c]
7.5	4	11.0±1.8	10.0±1.5	7.6±5.1	3.32±0.10 [b]	3.0±0.2	5.0
9.0	3	13.3±1.3	10.7±0.6	19.1±4.4	3.40±0.09 [b]	2.7±0.4	3.1
12.0	4	13.8±0.8	12.8±1.1	7.5±5.5	3.36±0.18 [b]	3.2±0.6	15.2 [c]
Dosed d11-13							
4.5	5	13.2±1.3	12.0±0.9	7.8±4.8	3.32±0.22	3.1±0.3	10.7
6.0	3	12.7±0.7	12.3±0.9	2.8±2.8	3.49±0.01	3.0±0.3	5.4
7.5	3	14.0±1.2	11.7±0.4	15.5±7.8	3.34±0.14 [b]	2.5±0.4	8.6
9.0	4	13.8±1.1	12.3±0.9	10.8±1.7	3.38±0.10 [b]	2.5±0.2	4.1

[a] Kidney score is a measure of renal papilla growth. Score of 1 represents complete growth of the papilla into the renal pelvis, 4 represents no down growth of the papilla, leaving a fully dilated renal pelvis and 2 and 3 are intermediate. The scores from both kidneys are combined thus minimum score =2, and maximum =8

[b] Significantly different from controls by ANOVA, $p \leq 0.05$

[c] Significantly different from controls by Fisher's exact test, $p \leq 0.05$

Table 3: Urinary Parameters in suckling rats prenatally exposed to dinoseb

Age (days)	Dose (mg/kg/day)	N	Body wt (g)	Kidney wt (mg)	Kidney wt/ body wt (%)	Urine osmolality (mOsm/kg) Basal	Urine osmolality (mOsm/kg) After 4 hr isolation	Urine volume (µl/g b.w./hr)	Urine pH	Chloride excretion (nEq/g b.w./hr
2	0	35	6.7±0.2	80±2	1.19±0.02	264±13	385±14	2.8±0.1	5.56±0.08	191±21
	8.0	32	6.8±0.1	78±2	1.15±0.02	246±10	414±11	2.3±0.1 [a]	5.45±0.05	58±3
	10.5	12	6.4±0.2	75±2	1.18±0.03	307±36	427±18	2.0±0.2 [a]	5.22±0.11 [a]	ND
15	0	34	30.6±0.4	378±6	1.24±0.02	310±19	735±28	1.8±0.1	6.31±0.05	19±3
	8.0	28	31.3±0.6	378±9	1.21±0.02	275±28	757±43	2.0±0.1	6.34±0.10	14±3
	10.5	8	31.1±0.4	389±8	1.25±0.03	238±9	685±53	1.6±0.1	6.19±0.08	14±4

[a] Significantly different from controls by general linear models procedure, $p \leq 0.05$

Table 4: Urinary parameters and response to 24 hr of water deprivation in PD30 rats prenatally exposed to dinoseb

	Dose (mg/kg/day)		
	0	8.0	10.5
N	44	39	13
Body weight (g)	74.4±1.2	85.6±1.4 [a]	62.0±3.1 [a]
Kidney wt (mg)	877±15	974±19	786±28
Kidney wt/body wt (%)	1.18±0.01	1.14±0.01	1.30±0.04 [a]
Urine osmolality (mOsm/kg)			
Normal	1387±58	1354±47	1369±65
Hydropenic	2142±70	2158±65	2141±111
Urine volume (µl/g b.w./day)			
Normal	89±4	71±2	99±6
Hydropenic	44±2	36±1	49±4
Urine pH			
Normal	7.75±0.07	7.87±0.06	7.59±0.17
Hydropenic	7.24±0.07	7.11±0.05	7.39±0.14
Urinary chloride excretion (µEq/g b.w./day)			
Normal	20.5±0.7	17.1±0.14	21.2±1.1
Hydropenic	14.8±0.4	13.2±0.5	17.2±1.4 [a]
Urinary sodium excretion (µEq/g b.w./day)			
Normal	10.7±0.4	10.2±0.4	ND [b]
Hydropenic	10.2±0.4	10.0±0.4	ND
Urinary potassium excretion (µEq/g b.w./day)			
Normal	22.3±0.7	21.2±0.7	ND
Hydropenic	20.2±0.6	20.0±0.8	ND

[a] Significantly different from controls by general linear models procedure, p≤0.05
[b] ND, not determined

Table 5: Maximal urine concentration ability after DDAVP challenge in suckling rats prenatally exposed to dinoseb

Age (days)	Dose (mg/kg/day)	N	Body wt. (g)	Urine volume (µl/g body weight/hr) 90 (minutes after initial void)	180	240	300	Urine osmolality (mOsm/kg) 0	90 (minutes after initial void)	180	240	300
6	0	18	14.2±0.6	3.9±0.5	1.9±0.3	0.6±0.1	0.6±0.1	228±18	421±38	708±34	860±26	915±25
	8.0	16	14.6±0.4	3.4±0.3	1.9±0.1	0.8±0.1	0.6±0.1	338±36 [b]	438±20	639±25	854±24	931±21
	10.5	4	13.0±0.3	1.7±0.1	1.5±0.1	0.9±0.3	1.1±0.6 [b]	282±10	599±50	684±22	766±16	816±33
14	0	17	27.7±0.6	3.4±0.4	1.2±0.1	0.6±0.1	0.7±0.1	292±26	417±47	790±33	989±34	1025±30
	8.0	16	30.2±1.4 [b]	2.6±0.4	0.8±0.1	0.6±0.1	0.6±0.1	232±17	612±54 [b]	946±25 [b]	1109±22 [b]	1157±18 [b]
	10.5	4	29.1±0.6	3.3±0.5	1.5±0.4	0.8±0.1	0.8±0.1	385±74	347±63	687±71	869±61 [b]	901±36 [b]

[a] These samples were collected after DDAVP injection and represent maximal urine concentrating ability and water conservation
[b] Significantly different from controls by general linear models procedure, p≤0.05

Table 6: Renal cortical function in rats prenatally exposed to dinoseb

Age (days)	Dose (mg/kg/day)	N	Slice/medium ratio (dpm/mg/dpm/µl)			
			PAII	TEA	AIB	MGP
3	0	16	8.01±0.29	5.23±0.22	7.36±0.28	0.87±0.06
	8.0	13	7.69±0.23	4.67±0.21	8.34±0.63	0.88±0.06
	10.5	6	9.62±0.60 [a]	5.60±0.54	7.35±0.16	0.86±0.17
17	0	30	6.56±0.25	6.02±0.14	5.49±0.26	1.03±0.06
	8.0	25	6.16±0.27	6.40±0.18	5.02±0.17	0.89±0.02
	10.5	8	6.49±0.36	5.76±0.21	6.05±0.34	0.99±0.11
30	0	26	5.73±0.21	7.72±0.27	2.70±0.25	0.75±0.04
	8.0	26	4.77±0.21 [a]	8.17±0.28	3.64±0.39	0.84±0.03
	10.5	8	7.30±0.65 [a]	7.90±1.02	2.36±0.51	0.72±0.06

[a] Significantly different from controls by general linear models procedure, p≤0.05

Table 7: Serum parameters in PD30 rats prenatally exposed to dinoseb

	Dose (mg/kg/day)	
	0	8.0
N	44	39
Sodium (mEq/l)	148±1	147±1
Potassium (mEq/l)	5.6±0.1	5.7±0.2

Urea nitrogen (mg/dl)	13±1	13±1
Creatinine clearance (µl/g b.w./day)	8140±98	7830±206
Urea nitrogen clearance (µl/g b.w./day)	5631±100	4974±146

Reliability : (2) valid with restrictions
06.03.2003 (5)

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